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Topical Review

Biosynthesis and Metabolic Degradation of Receptors for Epidermal Growth Factor

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The Linear Sequence of the EGF Receptor Gene Can Be Correlated with Functional Domains of the Receptor

The receptor for epidermal growth factor (EGF) is an integral membrane protein that mediates the mitogenic response of target cells to EGF and EGF-like polypeptides (Carpenter, 1984). The EGF receptor (EGF-R) has been purified from cultured human tumor cells (Cohen, Carpenter & King, 1980; Cohen, Ushiro, Stoscheck & Chinkers, 1982*b*) and mouse liver (Cohen, Fava & Sawyer, 1982*a*). The receptor is a glycoprotein having an estimated molecular mass of approximately 170,000 daltons and no subunit structure. In addition to its ligand-binding capacity, the receptor also acts as an allosteric enzyme; it contains a tyrosine-specific protein kinase activity that is activated by the binding of EGF (Carpenter, King & Cohen, 1979; Ushiro & Cohen, 1980). However, substrates of this receptor-kinase that are physiologically relevant to mitogenesis have not yet been identified.

The structure of the EGF receptor is schematically depicted in Fig. 1. The protein part of the mature receptor is a single polypeptide chain of 1186 amino acid residues. The complete amino acid sequence has been deduced from nucleotide sequencing of *c*DNA clones (Ullrich et al., 1984). Based on this linear, one-dimensional representation of the EGF receptor, the molecule can be subdivided into three domains. Residues 1 through 621 comprise the presumptive EGF-binding domain of the receptor, which is located on the extracellular face of the plasma membrane. The extracellular domain is characterized by a high content of cysteine, approximately 9%, and contains 12 of the 15 canonical sites (ASN-X-SER/THR) for potential N-linked glycosylation that are found within the entire receptor sequence. As discussed below, the EGF receptor molecule contains approximately 11 N-linked oligosaccharide chains, and these are most likely localized to this amino terminal external domain of the protein.

A second domain is formed by residues 622–644. The strongly hydrophobic nature of this sequence is typical of the transmembrane segment of many intrinsic membrane proteins. It is assumed that this is the only point at which the EGF receptor crosses the membrane. However, this point has not been confirmed, as yet, by direct experimental evidence. Recently, Criado et al. (1985) have provided evidence for the presence of unpredicted transmembrane domains in acetylcholine receptor subunits.

The third domain of the EGF receptor contains the carboxy-terminal 542 amino acid residues. This domain encodes the tyrosine kinase active site and is thought to be localized on the cytoplasmic face of the plasma membrane. The molecular mechanism by which EGF binding to the external domain modulates the activity of the protein kinase on the cytoplasmic domain is not known. Some of the features of this domain have, however, been elucidated. Tyrosine residues that are autophosphorylated by the kinase have been mapped at residues 1068, 1148 and 1186 (Downward et al., 1984). The lysine at residue 721 is covalently labeled with *p*-fluorosulfonylbenzoyl-adenosine (FSBA), an ATP analogue (Russo, Lukas, Cohen & Staros, 1985) and, thus, would appear to be at or very near the ATP binding site for the kinase. When cultured cells are incubated with $^{32}P_i$, the EGF receptor contains not only radioactive tyrosine residues, but also radioactive serine and threonine residues. The phosphorylation of serine and threonine residues is not the result of receptor-kinase autophosphorylation.

Key Words EGF receptor · glycoprotein synthesis · N-linked oligosaccharides · EGF receptor turnover · tyrosine kinase · A-431 cells

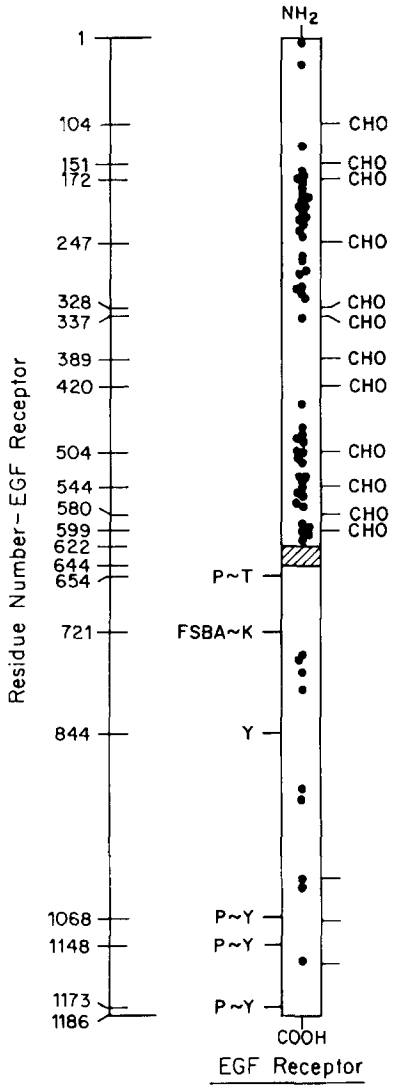

Fig. 1. Structural map of the EGF receptor. The deduced amino acid sequence data of Ullrich et al. (1984) was used to position cysteine residue (●); all canonical sites for potential N-linked glycosylation are indicated (—) and those that are likely to actually be utilized are designated (—*CHO*); the transmembrane sequence is identified by crosshatches. Known sites of autophosphorylation (Downward, Parker & Waterfield, 1984) are indicated by *P ~ Y*. The C kinase-induced phosphorylation of a threonine residue (Hunter et al, 1984; Davis & Czech, 1985) is shown as *P ~ T*. The lysine residue is labeled with *p*-fluorosulfonylbenzoyl adenosine (Russo et al., 1985) is designated *FSBA ~* K. At residue 844, *Y* indicates the tyrosine residue that is homologous to the phosphorylation site of the *src* family of kinases

While the location of these phosphorylation sites are mostly unknown, one threonine phosphorylation site, at residue 654, has been identified (Hunter, Ling & Cooper, 1984; Davis & Czech, 1985). The data indicate that this threonine phosphorylation is attributable to the activity of protein kinase C and may induce functional modulation of receptor binding and tyrosine-kinase activity.

The cytoplasmic domain of the EGF receptor exhibits considerable sequence homology with other tyrosine kinases (for reviews *see* Staros, Cohen & Russo, 1985; Hunter & Cooper, 1985). The tyrosine at residue 844 is analogous to the tyrosine site of autophosphorylation in the *src* kinase, although this residue has not been identified as an autophosphorylation site in the native EGF receptor. Large portions of the EGF receptor cytoplasmic domain have an unexpectedly high level of homology to one other tyrosine kinase in particular, the protein product of the *erb B* oncogene from avian erythroblastosis virus (Downward et al., 1984; Ullrich et al., 1984). The v-*erb B* protein seems to be a truncated version of the EGF receptor, corresponding to residues 557 through 1160 of the EGF receptor. Phosphorylation activity in the *erb B* protein has only recently been demonstrated (Decker, 1985; Kris et al., 1985; Gilmore, DeClue & Martin, 1985).

A major tool in elucidating the characteristics and structure of the EGF receptor has been a particular cell line, the human epidermoid cacinoma A-431 cell. The value of this cell derives from its exaggerated production of EGF receptors. The A-431 cell contains approximately 3×10^6 EGF receptors per cell (Haigler, Ash, Singer & Cohen, 1978), a value that is 20- to 50-fold higher than the number of receptors found in other cell lines. A review of this cell line has been published elsewhere (Stoscheck & Carpenter, 1983). Many of the studies described below were performed in A-431 cells. The reader should keep in mind that this cell line may yield details of receptor physiology that are not representative of most cells, although the essential characteristics have proven accurate so far.

The EGF Receptor Gene Codes for More Than One mRNA

The gene for the EGF receptor has been mapped to human chromosome 7 in the p13–q22 region (Kondo & Shimizu, 1983). The gene for EGF maps to human chromosome 4 (Brissenden, Ullrich & Francke, 1984; Zabel et al., 1985), while the gene for the EGF-like polypeptide transforming growth factor-α has not been localized. Sequencing studies of the EGF receptor gene have not yet been reported, so the organization of exon and introns and nontranscribed controlling regions is not known.

Due to the high level of sequence homology between the EGF receptor and the v-*erb B* protein,

it has been postulated that the gene for EGF receptor may be the cellular gene from which the v-*erb B* oncogene was derived. Consistent with this notion, a c-*erb B* probe hybridizes to the p7–q22 region of human chromosome 7 (Zabel et al., 1984; Spurr et al., 1984).

Recent studies by several laboratories (Ullrich et al., 1984; Xu et al., 1984*a*,*b*; Lin et al., 1984; Merlino et al., 1984) have utilized cDNA probes to detect EGF receptor mRNA on Northern blots. The results show that in most all mammalian cells mRNA molecules of approximately 10.5 and 5.8 kb specifically hybridize with the EGF receptor probes. In some tumor cells, particularly the A-431 human epidermoid carcinoma cell line, a smaller mRNA of approximately 2.8 kb is also detected. One can calculate that production of the EGF receptor protein (1210 amino acids including the post-translationally hydrolyzed signal sequence) would require a mRNA molecule of at least 3.6 kb. The 2.8-kb mRNA species, therefore, would not appear to be involved in synthesis of the 170,000-dalton native receptor molecule. Rather, this mRNA molecule seems to be associated with the production of a truncated form of the EGF receptor that is produced in certain tumor cells and secreted into the media (*see below*).

While it has not been possible to translate EGF receptor mRNA's in vitro, mRNA from A-431 cells has been translated after microinjection into *Xenopus laevis* oocytes (Simmen et al., 1984*b*). When the mRNA preparation was size fractionated on a sucrose gradient and the fraction containing the two larger mRNA species was microinjected into *Xenopus* oocytes, a full-size EGF receptor was recovered by specific immunoprecipitation. RNA from the gradient fractions containing the 2.8 kb mRNA species also was translated in *Xenopus* oocytes and yielded an EGF receptor-related glycoprotein of approximately 100,000 daltons. In these studies it was not determined whether the translation of EGF receptor mRNA in *Xenopus* oocytes gave rise to functional EGF receptor molecules or whether these molecules were inserted into the oocyte plasma membrane.

The relationship of the two larger receptor mRNA species remains unclear. One cannot be confident, based on available data, that the 10.5 and 5.8 kb mRNAs code for precisely the same gene product. One could code for the EGF receptor and the other for a related protein, such as the *neu* oncogene product (Schechter et al., 1984). It is more likely, however, that these RNA molecules result from the use of different polyadenylation sites during the transcription of one gene. Obviously, alternative splicing mechanisms could generate the 10.5 and 5.8 kb mRNA's from a single transcript of the EGF receptor genes. Aberrant splicing might also generate the smaller 2.8-kb mRNA in tumor cells.

Essentially nothing is known about factors that regulate the transcription of the EGF receptor gene. In certain tumor cells there is enhanced transcription of EGF receptor sequences (Ullrich et al., 1984; Xu et al., 1984*a*; Simmen et al., 1984*a*; Merlino et al., 1984; Lin et al., 1984; Lieberman et al., 1985). In part, this enhanced transcription is due to amplification of the EGF receptor gene and possibly to rearrangement of the receptor gene. A causal relationship between these events and the biology of these tumor cells is possible, but has not been substantiated.

The EGF Receptor is Synthesized as a 160,000-Dalton Precursor

The mRNA's for integral membrane proteins are translated on polysomes bound to the cytoplasmic surface of the rough endoplasmic reticulum (ER). These translation products typically contain at the amino terminus a signal sequence that allows the nascent peptide chain to traverse the lipid bilayer of the ER. Once the nascent polypeptide is extruded into the lumen of the ER, the signal sequence is proteolytically removed.

N-linked glycosylation of the protein also occurs within the lumen of the ER. The high-mannose core structure common to all N-linked oligosaccharides is synthesized by stepwise addition of the appropriate monosaccharides to a dolichol phosphate intermediate. The oligosaccharide core is then transferred *en bloc* to the amide nitrogen of asparagine residues in canonical Asn-X-Ser/Thr sequences along the polypeptide. The resulting structure is depicted in the diagram labeled "precursor" in Fig. 2.

N-linked glycosylation, thus, occurs co-translationally. It is not clear, however, whether glycosylation sites are utilized in a linear order, beginning with the site nearest the amino terminus of the protein. Certainly, not all potential sites are necessarily used. It is probable that folding of the protein occurs very rapidly, causing steric hindrance that interferes with glycosylation at certain sites.

Carbohydrate can also be added to proteins in O-linkage to the hydroxyl side chains of serine or threonine residues. O-linked oligosaccharides, however, are added to proteins post-translationally, within the Golgi.

When cells are pulse-labeled with ^{35}S-methionine for a short period of time, an EGF receptor molecule of 160,000 daltons (160K) is de-

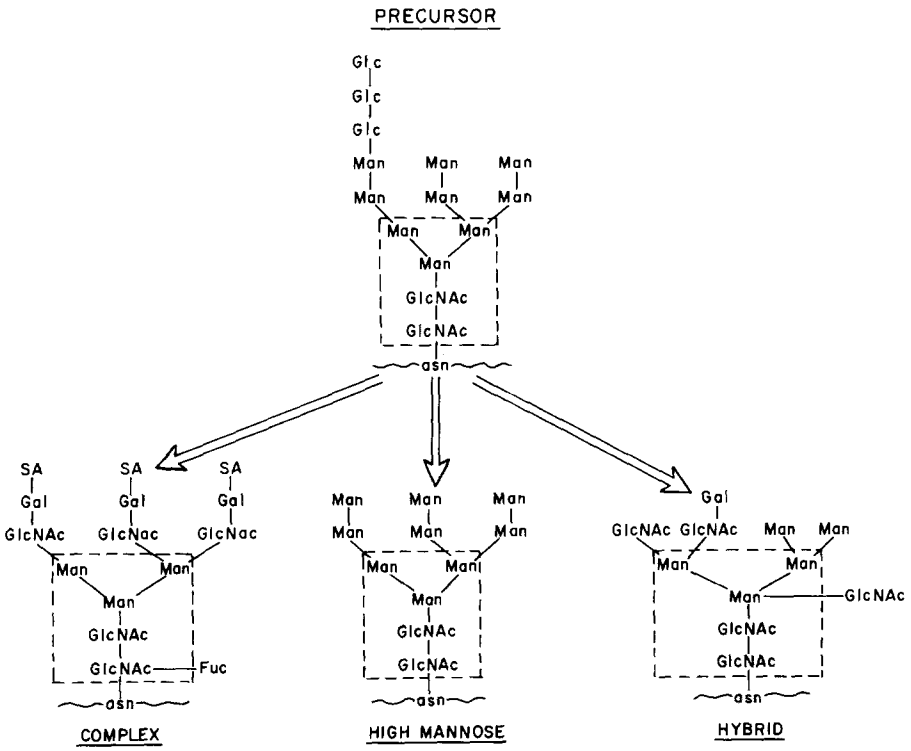

Fig. 2. Structure of N-linked oligosaccharide side chains present on the primary precursor of glycoproteins and representative structures of N-linked oligosaccharide chains found on mature glycoproteins. The core oligosaccharide unit, which is not altered during processing, is enclosed by the dotted lines. The structures are adapted from Snider (1984)

tected by immunoprecipitation (Mayes & Waterfield, 1984; Soderquist & Carpenter, 1984; Stoscheck, Soderquist & Carpenter, 1985; Decker, 1984*a*,*b*; Carlin & Knowles, 1984; Slieker & Lane, 1985). In the A-431 tumor cell line, a protein of 90,000 daltons is also detected, but this protein is peculiar to this cell line and is not involved in the biosynthesis of the mature, plasma membrane receptor (*see below*). EGF does not modulate the rate of production of the receptor protein (Stoscheck et al., 1985), although it does affect the metabolic rate of receptor degradation (*see below*).

The 160K protein can be labeled with ^{3}H-glucosamine and, to a greater extent, with ^{3}H-mannose (A.M. Soderquist & G. Carpenter, *unpublished results*). Both sugars are present in high-mannose N-linked oligosaccharide cores (*see* Fig. 2). The 160K protein is also sensitive to endoglycosidase H (Endo H), an enzyme that removes these N-linked cores, as well as related high-mannose N-linked chains, from glycoproteins. Digestion of the pulse-labeled 160K protein with Endo H reduces its apparent molecular weight to 130K.

That the Endo H-sensitive, 160K protein is the precursor for the mature, 170K EGF receptor is substantiated by pulse-chase experiments. The 160K molecule immunoprecipitated from pulse-labeled cells is quantitatively converted to an immunoprecipitable 170K molecule when pulse-labeled cells are chased several hours with fresh medium. The 170K receptor species shows only limited sensitivity to Endo H, as would be expected if most of its N-linked oligosaccharide chains were processed to complex, Endo H resistant chains.

Interestingly, a recent report (Slieker & Lane, 1985) indicates that the newly synthesized 160K receptor precursor is not able to bind EGF. Rather, binding capacity is gradually acquired, beginning approximately 15–30 min after synthesis. The cause of this post-translational activation is not clear, but the authors consider it likely that disulfide bond formation and/or rearrangement is involved. In support of this suggestion, the amino terminal, ligand-binding domain of the EGF receptor contains a high content of cysteine residues—50 of the 59 cysteine residues in the entire receptor molecule. In addition, enzymes capable of forming or isomerizing disulfide bonds are located within the lumen of the endoplasmic reticulum. However, factors such as oligosaccharide processing, other post-translational modifications, or translocation to intracellular compartments beyond the ER might equally well be responsible for this activation.

Processing of N-linked Oligosaccharides is Responsible for the Conversion of the EGF Receptor Precursor to its Mature Form

Processing of the precursor N-linked oligosaccharide (Fig. 2) that is added co-translationally to proteins begins while the proteins are still within the

endoplasmic reticulum. Specific glycosidases remove the three glucose residues and at least one of the mannose residues. The partially processed glycoprotein then moves from the endoplasmic reticulum to the Golgi. Within the different compartments of the Golgi, additional mannose residues are cleaved. Golgi glycosyl transferases may then add outer sugar residues, such as fucose, N-acetyl glucosamine, galactose, and sialic acid.

These processing steps can give rise to a large variety of N-linked oligosaccharide structures in mature glycoproteins. Representative examples are shown in Fig. 2. Most common of these structures are the complex type chains, which are characterized by an inner core of $Man_3GlcNAc_2$, with two to five branches containing sialic acid, galactose, and N-acetyl glucosamine. Complex chains can be crudely characterized by their resistance to Endo H. Less frequently, N-linked chains of the high-mannose type are found on mature glycoproteins. In contrast to complex type chains, the high mannose chains are sensitive to removal by Endo H. The third type of N-linked chain that may occur on mature glycoproteins is the hybrid type, in which one branch of the core oligosaccharide remains high-mannose, while the other is processed to complex-type. These hybrid oligosaccharide chains are also sensitive to Endo H.

The EGF receptor isolated from A-431 cells contains approximately 11 N-linked oligosaccharides (Mayes & Waterfield, 1984; Cummings et al., 1985). Details of the oligosaccharide structures present on the mature receptor in these cells have recently become available (Cummings, Soderquist & Carpenter, 1985). The data suggest that $\frac{2}{3}$, or about 7, of the N-linked chains are complex, while the remainder are high-mannose type. The complex chains contain relatively little sialic acid, but are highly fucosylated. These initial characterizations of the oligosaccharide chains present on the EGF receptor in A-431 cells cannot, as yet, be generalized to other cell types. The processing of oligosaccharide chains may differ somewhat in tumor versus normal cells or in cells from different tissues.

Atkinson and Lee (1984) have surveyed the distribution of complex and high-mannose type oligosaccharide chains as a function of their relative position on a linear map of known glycoproteins. Their conclusions are that complex chains are more likely to be found near the amino terminus of a glycoprotein, while high-mannose chains are more likely to be located nearer the carboxy terminus of the polypeptide chain. If the distribution of oligosaccharide chains on the EGF receptor follows this general rule, then the four high-mannose chains would be at those glycosylation sites on the external domain of the receptor that are closest to the transmembrane region. These sites on the EGF receptor would be analogous to the three glycosylation sites on the small external segment of the v-*erb B* oncogene product. However, in the v-*erb B* protein these sites would occur close to the amino terminus and would be expected to be of the complex type.

There have been recent reports of immunologic relatedness between the EGF receptor and blood group antigens (Fredman et al., 1984; Parker et al., 1984; Childs et al., 1984), which are carbohydrate sequences typically associated with O-linked oligosaccharides. Although no evidence has been found for O-linked oligosaccharides on the EGF receptor (Cummings et al., 1985; Soderquist & Carpenter, 1984), some of the branches of the complex, N-linked chains of the receptor terminate in N-acetylgalactosamine. This particular sugar is common to O-linked chains but seldom present on N-linked chains. The presence of this residue is likely to be responsible for the observed immunologic relatedness (Cummings et al., 1985).

Studies have been performed (again, in A-431 cells, which may not be representative of all cell types) to determine the time required for newly synthesized EGF receptors to be processed and inserted into the plasma membrane (Stoscheck et al., 1985). When cells are pulse labeled with ^{35}S-methionine for 15 min and the label is then chased for varying lengths of time, 3–4 hours are required before maximum labeled receptor is detected on the cell surface. This is a somewhat longer time than the 30–90 min usually quoted for the complete biogenesis of plasma membrane glycoproteins. Although the factors involved in their relatively slow biogenesis have not been described, there are at least two other glycoproteins, the acetylcholine (Merlie, 1984) and insulin (Ronnett, Tennekoon, Knutson & Lane, 1983) receptors, that require approximately 3 hr to reach their mature form. Kinetic measurements have shown that conversion of the 160K EGF receptor precursor to its 170K mature form is a slow process, the half-time of conversion in A-431 cells being approximately 1.7 hr (Stoscheck et al., 1985).

The EGF Receptor is Active Despite Alterations in Oligosaccharide Processing but Inactive in the Absence of Glycosylation

There are several inhibitors available that affect specific steps in the N-linked glycosylation of proteins. Some of these have been used to alter the glycosylation of the EGF receptor in A-431 cells (Soderquist & Carpenter, 1984).

The plant alkaloid, swainsonine, inhibits Golgi α-mannosidase II and, therefore, prevents removal

of mannose residues from one of the branches of the N-linked core structure (Tulsiani, Harris & Touster, 1982). In the presence of swainsonine, cells accumulate EGF receptor molecules in a partially processed form, i.e., all of the oligosaccharide chains are sensitive to Endo H (Soderquist & Carpenter, 1984). The receptor produced in swainsonine-treated cells has an apparent molecular mass of approximately 160,000 daltons (160K). Despite the similarity in molecular weight and Endo H sensitivity, the oligosaccharide structure of the receptor produced during swainsonine treatment should not be identical to that of the 160K receptor precursor previously described. The swainsonine-derived 160K receptor, in fact, seems to exhibit all of the functional properties of the mature 170K receptor. It is found on the plasma membrane, is capable of binding ^{125}I-EGF, has tyrosine kinase activity, and can mediate EGF-induced DNA synthesis (Soderquist & Carpenter, 1984).

Based on the data from swainsonine-treated cells, it would appear that complete processing of N-linked oligosaccharides is not necessary for receptor function. What function the terminal sugars on complex-type chains serve is not known, although it is possible that they protect the oligosaccharides from extracellular glycosidases.

The antibiotic, tunicamycin, inhibits the first step in the formation of the oligosaccharide-dolichol phosphate intermediate, thereby preventing the addition of N-linked oligosaccharide chains to proteins. Since there are no O-linked oligosaccharides on the EGF receptor (Soderquist & Carpenter, 1984; Cummings et al., 1985), incubation of cells in medium containing tunicamycin results in the synthesis of nonglycosylated receptor molecules (Decker, 1984*a*,*b*; Mayes & Waterfield, 1984; Soderquist & Carpenter, 1984; Slieker & Lane, 1985). On SDS-polyacrylamide gels this molecule migrates at an apparent molecular mass of 130,000 daltons (130K) which is quite close to the value (131,360 daltons) predicted from the cloned receptor sequence (Ullrich et al., 1984). The tunicamycin-derived receptor molecule also co-migrates on SDS gels with the 130K receptor produced by Endo H digestion of the receptor precursor (Soderquist & Carpenter, 1984).

In contrast to modification of oligosaccharide processing, prevention of N-linked oligosaccharide addition has a profound effect on EGF receptor function. The tunicamycin-derived 130K receptor is not translocated to the cell surface but accumulates, instead, in some intracellular compartment, probably the endoplasmic reticulum (Soderquist & Carpenter, 1984). This intracellular compartmentalization of receptor would account for the loss of ^{125}I-EGF binding activity seen in the past in glycosylation deficient 3T3 cells (Pratt & Pastan, 1978). However, not all glycoproteins whose glycosylation is inhibited fail to be properly translocated. Although the asialoglycoprotein receptor, for example, is synthesized as a nonglycosylated molecule in cells incubated with tunicamycin, it reaches the cell surface and functions as well as the fully glycosylated receptor (Breitfield, Rup & Schwartz, 1984).

The EGF receptor protein produced in the presence of tunicamycin has also been assayed for biochemical functions. Since the nonglycosylated receptor is not present on the cell surface, assays for ^{125}I-EGF binding capacity were carried out on detergent solubilized extracts (Soderquist & Carpenter, 1984). The ^{125}I-EGF binding capacity of extracts prepared from cells treated with tunicamycin is reduced to approximately 25% of the ligand binding capacity of untreated, control cells. Most or all of the residual activity in the tunicamycin-treated cells can be attributed to receptors synthesized prior to the addition of tunicamycin. The half-life for the EGF receptor in A-431 cells is 20 hr (Stoscheck & Carpenter, 1984*a*) and tunicamycin does not markedly affect receptor degradation (Soderquist & Carpenter, 1984). Based on this degradation rate, one would expect that approximately 15–20% of the receptors synthesized prior to treatment would not have been degraded during the 48-hr period of inhibitor treatment. The conclusion, therefore, is that nonglycosylated EGF receptors are not able to bind EGF. With a somewhat different assay for EGF binding activity, Slieker and Lane (1985) reached the same conclusion.

Studies have also been carried out to determine whether the EGF receptor's tyrosine kinase activity, as measured by autophosphorylation of the receptor molecule, is affected by the inhibition of glycosylation (Soderquist & Carpenter, 1984). The carboxy terminal kinase domain of the receptor is not expected to enter the lumen of the endoplasmic reticulum, but should remain exposed to the cytoplasm during receptor biogenesis. Although incorrect folding of the amino terminal portion of the receptor would be a possible, if not likely, consequence of glycosylation inhibition, one would not necessarily expect that incorrect folding of the amino terminal domain would affect the carboxy terminal. However, the experimental results indicate that the absence of carbohydrate does affect the EGF receptor kinase activity. The 130K aglycoreceptor is not phosphorylated when extracts from tunicamycin-treated cells are subjected to in vitro phosphorylation and immunoprecipitation. A protein of approximately 140,000 daltons (140K) is phosphorylated and specifically immunoprecipi-

tated from these extracts, but control experiments have shown that this 140K phosphoprotein is not a phosphorylated form of the 130K nonglycosylated receptor. Rather, the 140K receptor species seems to be a partially glycosylated form of the EGF receptor that is produced at low levels despite the presence of tunicamycin. These low levels have made it impossible thus far to characterize the carbohydrate present on this species. In light of the apparent biochemical activity present in this partially glycosylated receptor molecule, it will be extremely interesting, however, to uncover more details about its structure.

A-431 Cells Synthesize a Truncated EGF Receptor Protein that is Secreted

As mentioned above, a truncated form of the EGF receptor molecule is produced by the human A-431 tumor cell line. It is likely that this truncated molecule is translated from the small 2.8 kb mRNA species that is found in these tumor cells. In tunicamycin treated A-431 cells, a shortened EGF receptor protein of approximately 68,000 daltons is found in addition to the full length, 130K, nonglycosylated receptor protein (Soderquist & Carpenter, 1984; Stoscheck et al., 1985). In pulse-labeling studies of untreated cells this aberrant EGF molecule is glycosylated co-translationally and has an apparent molecular mass of about 95,000 daltons. While oligosaccharide processing results in a mature species of approximately 110,000 daltons, the mature molecule is not inserted into the plasma membrane. Instead, it is secreted from the cell and accumulates in the medium. Since this molecule is glycosylated similarly to the 170K receptor and binds ^{125}I-EGF, although with low affinity, it would seem to be related to the external domain of the mature EGF receptor. In fact, both molecules have identical N-terminal amino acid sequences (Weber, Gill & Spiess, 1984). That the truncated receptor is secreted would suggest that it lacks the transmembrane sequence of the mature molecule. This protein probably corresponds to the cDNA clone described by Ullrich et al. (1984), that codes for a truncated receptor sequence homologous to the external domain of the EGF receptor. Unfortunately, no physiological role can be assigned to this unusual form of the EGF receptor at this point.

EGF Receptor Turnover is Enhanced by Exogenous EGF

Within the last two years several groups have made direct measurements of the rate of EGF receptor degradation (Beguinot, Lyall, Willingham & Pustan, 1984; Carpenter, 1984*a*,*b*; Cooper, Scolnick, Ozanne & Hunter, 1983; Decker, 1984*a*,*b*; Stoscheck & Carpenter, 1984*a*,*b*). These studies have utilized a protocol in which cells are first metabolically labeled with ^{35}S-methionine. The medium is then replaced with medium containing nonradioactive methionine, and at various times during this "chase" the cells are lysed. Antibodies to the EGF receptor are then used to specifically precipitate the receptor. Subsequently, the receptor is analyzed by SDS gel electrophoresis, and the amount of radiolabeled receptor present is quantitated by fluorography and densitometric scanning. Previous studies had indirectly estimated receptor turnover rates using inhibitors and measurements of ^{125}I-EGF binding activity (Aharonov, Pruss & Herschman, 1978; Carpenter, 1979; Bhargava & Makman, 1980; King, Willis & Cuatrecasas, 1980). Another study utilized density-shift techniques in place of inhibitors, but still relied on ^{125}I-EGF binding to locate and quantitate the receptor in different gradient fractions (Krupp, Connolly & Lane, 1982). Although the results from these different approaches are not grossly different, the data from more direct metabolic labeling and immunoprecipitation studies are likely to be the most accurate.

In human fibroblasts, the EGF receptor has a half-life of approximately 10 hr when the measurements are made in cells cultured in the absence of exogenous EGF (Stoscheck & Carpenter, 1984*a*). Receptor turnover under these conditions is significantly faster than turnover of total cell protein ($t_{1/2}$ = 51 hr). When EGF is added to fibroblast cultures, there is a marked change in the rate of receptor degradation. In the presence of EGF, but not other growth factors, the EGF receptor is degraded with a half-life of 1 hr. The EGF-induced accelerated degradation of its receptor is prevented by chloroquine, methylamine, reduced temperature (25°), or iodoacetate, all of which are known to interfere with either the translocation of endocytic vesicles to lysosomes or with lysosomal function. It would appear, therefore, that upon the addition of EGF to cultured cells, EGF:receptor complexes are formed at the surface, these complexes are rapidly internalized in endocytic vesicles, and ultimately (and perhaps coordinately) both the ligand and receptor are degraded within lysosomes. Potential intracellular processing and translocation of the tyrosine kinase domain of the EGF receptor, subsequent to internalization, has presented an attractive mechanism for nuclear signalling. Unfortunately, the studies described above have not provided evidence to support such a mechanism. None of the reported investigations has been able to dem-

onstrate the production of lower molecular weight receptor fragments. It is possible, however, that processing occurs, but involves only a small fraction of the total receptor molecules, below the levels of detection in these experiments.

EGF Receptor Synthesis is Important in the Biological Response of Cells to the Growth Factor

Carpenter and Cohen (1976) demonstrated that quiescent cells had to be exposed to EGF for a substantial period of time for DNA synthesis to be stimulated. In these experiments human fibroblasts were exposed to EGF for 12 hr before an increase in ^{3}H-thymidine incorporation into DNA was apparent. Maximal stimulation of DNA synthesis occurred after a 20-hr exposure to EGF. To explore the growth factor requirement during the 12-hr lag between addition of EGF and the beginning of increased DNA synthesis, specific antibodies to EGF were added to separate cultures at different times during this 12-hr interval. This maneuver effectively prevented the exogenous EGF in the medium from interacting with receptors on the cell surface. The results showed that addition of the anti-EGF within 6–8 hr after the addition of EGF completely prevented a stimulation of DNA synthesis. This result has been confirmed by others, with nearly identical results (Lindgren & Westermark, 1976, 1977; Schechter, Hernaez & Cuatrecasas, 1978; Haigler & Carpenter, 1980; Das, 1981). Since the binding of EGF to the cell surface is rapid, as are internalization and degradation of the hormone : receptor complexes, cells would need to synthesize new receptor molecules during the 6–8 hr commitment period to enable continuous ligand-receptor interactions to occur at the cell surface. This indicates that receptor biosynthesis is a potential control point in the regulation of cellular transit from a quiescent state to a proliferative state.

Clearly, the capacity of a cell to respond to any particular extracellular polypeptide hormone or growth factor is dependent on the expression of the appropriate membrane receptor. However, the formation of ligand : receptor complexes on the cell surface, while necessary, is clearly not sufficient for the production of biological responses. A major task at this point in the growth regulation field is to identify the biochemical nature of the steps subsequent to ligand binding that mediate the activity of growth signals such as EGF.

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