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EGF stimulates the processing and export of a secreted form of EGF receptor

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Human A431 cells express a 100 kDa EGF-receptor homolog that contains only the external domain. The kinetics of its maturation and export are slow and comparable to those of the transmembrane receptor. Here we demonstrate that exogenously added EGF stimulates post-translational processing and export of this receptor through a pathway that is insensitive to inhibitors of protein synthesis. The results suggest that EGF may influence one or more of the rate determining steps that control receptor export from endoplasmic reticulum. This may constitute one of the pathways by which EGF regulates the expression of its receptor.

EGF receptor; Glycoprotein processing; Protein secretion; Biosynthetic transport

1. INTRODUCTION

The epidermal growth factor (EGF)-receptor belongs to a group of transmembrane tyrosine kinases that includes as its members the *neu* protein and the receptors for insulin and insulin-like growth factors [1]. The external domains of these proteins contain two structural features – the cysteine-rich regions and the sites for N-linked glycosylation – that are important from the viewpoint of post-translational maturation and externalization to the cell surface [1–4]. In this paper we describe the processing and export characteristics of a secreted 100 kDa homolog of EGF-receptor, which is identical to the external domain of the transmembrane receptor in its primary structure [5]. The results indicate that the processing and export characteristics of the soluble receptor are similar to those of the membrane anchored form. Moreover we find that EGF, the ligand, accelerates post-translational processing and secretion of the 100 kDa glycoprotein. These effects occur independent of any EGF-induced stimulation of receptor protein synthesis.

2. MATERIALS AND METHODS

A431 cells were grown as described in Bulbecco's modified Eagle's (DME)-medium containing 7% fetal bovine serum (FBS) [9,11].

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Abbreviations: EGF, epidermal growth factor; DME-medium, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; ER, endoplasmic reticulum

Western blotting was conducted as described using an EGF-receptor specific rabbit polyclonal antibody and ^{125}I-labelled protein A [6,7]. Immunoprecipitation was conducted as described using a mouse monoclonal anti-EGF-receptor antibody [8,9].

3. RESULTS AND DISCUSSION

3.1. *EGF increases the steady state level of EGF-receptor in the medium*

The studies described here were conducted with human A431 cells – a cell type that expresses both the kinase active (EGF-responsive) transmembrane 170 kDa receptor and the kinase domain deficient secreted 100 kDa homolog. The results in Fig. 1 show that exposure of cells to EGF results in increased accumulation of the 100 kDa receptor in the medium. This EGF-induced increase is blocked by monensin but not by chloroquine or cycloheximide. These results suggest that ongoing protein synthesis is not required for the effect, i.e. the majority of the 100 kDa receptors released in response to EGF are pre-synthesized proteins blocked in a pre-Golgi compartment.

3.2. *EGF enhances the secretion of pre-synthesized receptors*

To dissociate the effect of EGF upon receptor maturation/export from its effects on receptor protein synthesis, pulse-chase studies were conducted. In these studies, cells were labelled with [^{35}S]methionine for 1 h, then washed free of labelled proteins and incubated with unlabelled medium containing cycloheximide for different time periods (Fig. 2). Release of labelled receptor into the medium was analyzed by immunoprecipitation. The results show that there is time-dependent release of pre-synthesized labelled 100 kDa

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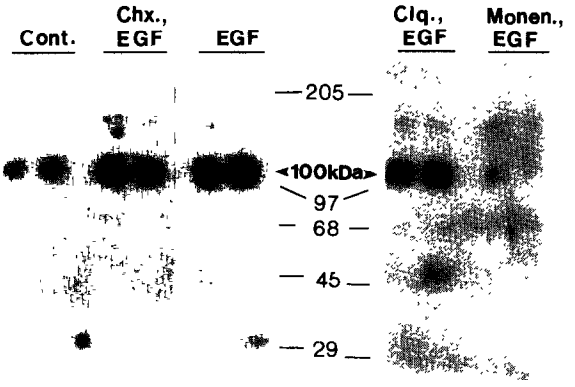

Fig. 1. EGF increases the steady-state level of 100 kDa receptor in the medium. A431 cells in 60 cm dishes were washed with serum-free DME-medium and then incubated at 37°C for 6 h with 3 ml of the same medium containing 20 nM EGF, 10 μg/ml cycloheximide (Chx.), 100 μM chloroquine (Clq.), 10 μM monensin (Monen.), or no addition (Cont.). At the end of incubation, cellular media were subjected to Western blot analysis.

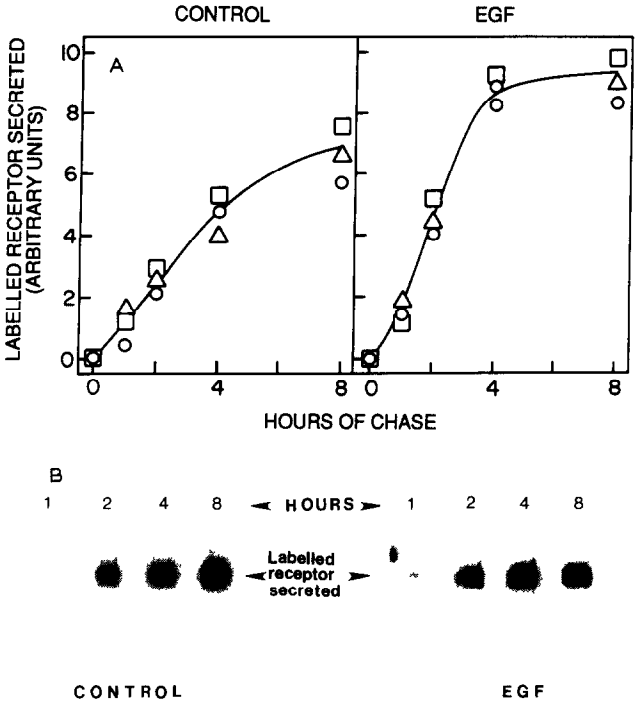

Fig. 2. Effect of EGF on the time-course of secretion of presynthesized 100 kDa receptor. In three seperate experiments (○, △, □), cells in 16 mm dishes were incubated for 1 h with [^{35}S]methionine (100 μCi/ml) as described [9], then chased at 37°C for the indicated times in the presence and absence of 20 nM EGF in DME-medium containing 0.1% bovine serum albumin and 10 μg/ml cycloheximide. Aliquots of cellular media were subjected to immunoprecipitation and radiolabelled receptor was quantified by densitometric analysis of the autoradiograms (see panel A). Autoradiogram for one of the experiments (△) is shown in panel B. Acid-precipitable radioactivity in media (overall protein secretion) determined as described [9], showed no difference between control and EGF samples.

receptor into the medium. The rate of release is slow ($t_{1/2} \geq 4$ h), but is enhanced in the presence of EGF ($t_{1/2} \approx 2$ h) (Fig. 2). The EGF-induced effect was specific for the 100 kDa receptor, i.e. the rate of overall protein secretion ($t_{1/2} \approx 1.5$ h) was not significantly altered by EGF.

3.3. *Effects of glycosylation inhibitors on receptor export*

The appearance of labelled 100 kDa receptor in the medium is inhibited when tunicamycin [10] is added during cell labelling, but not when it is added during chase (Fig. 3). Similar experiments with deoxynojirimycin [10] showed that it inhibits labelled receptor export irrespective of whether it is added during labelling or during chase (Fig. 3). In contrast, the presence of swainsonine [10] during labelling or chase does not affect receptor export, only the product released is smaller than the fully processed glycoprotein (Fig. 3).

Previous studies with the intact 170 kDa EGF receptor had indicated that both tunicamycin and deoxynojirimycin inhibit the exit of the protein from the endoplasmic reticulum (ER) [1,3]. The results described here for the soluble receptor suggest that the biosynthetic transport pathway of the isolated external domain may not differ much from that of the intact receptor, despite the fact that the intact receptor is membrane-anchored and the 100 kDa receptor is free-floating in the lumen of ER. Overall the results in Fig. 3 indicate that tunicamycin-sensitive core glycosylation of the 100 kDa receptor occurs soon after its entry into the lumen of ER, but the subsequent modifications (e.g. the deoxynojirimycin-sensitive processing within ER) which lead to its export from ER are slow and rate determining from the viewpoint of secretion.

3.4. *EGF accelerates receptor processing*

Next we tested whether EGF accelerates the rate-determining events in receptor export. Cells were labelled with ^{35}S-methionine, then chased with unlabelled medium containing EGF or no EGF with deoxynojirimycin being added at various time points during chase. Since receptors that do not undergo the deoxynojirimycin-sensitive processing are not exported, the experiment in Fig. 4 provided a means for studying the time-course of this processing. The results show that the step is slow, but EGF accelerates it by 3–4-fold.

Similar analysis was conducted for the swainsonine-sensitive event. The swainsonine-sensitive conversion of the high-mannose immature receptor to the mature 100 kDa receptor was found to require ≈4 h for completion in the absence of EGF, but only ≈2 h when EGF was present (data not shown).

In previous studies we showed that EGF regulates syntheses and stabilities of EGF receptor mRNA and protein in A431 cells [9,11]. EGF also enhances the level of the 2.6 kb mRNA that encodes for the 100 kDa solu-

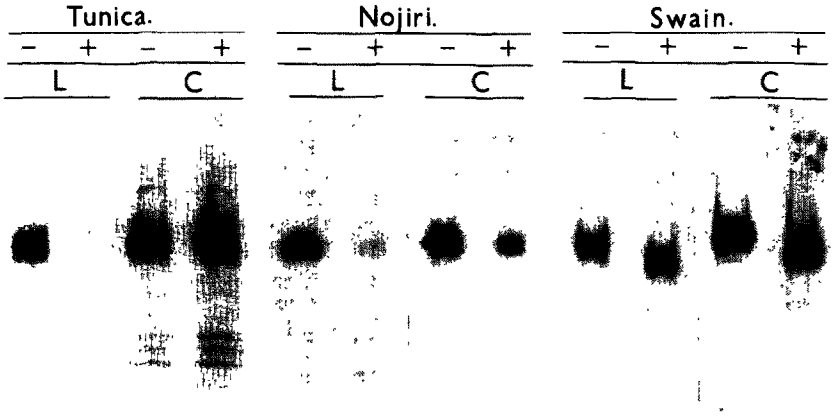

Fig. 3. Effect of glycosylation inhibitors on secretion of 100 kDa receptor. In experiments where inhibitors were present during cell labelling (lanes marked L), A431 cells were labelled with [^{35}S]methionine for 4 h in the presence (+) or absence (−) of 1 μg/ml tunicamycin (Tunica), 4 mM deoxynojirimycin (Nojiri), or 1.5 μg/ml swainsonine (Swain.), and then media were subjected to immunoprecipitation. In experiments where the inhibitors were present during the chase (lanes marked C), cells were labelled with [^{35}S]methionine for 4 h, then washed, chased at 37°C for 11 h with DME-0.1% BSA medium in the presence (+) or absence (−) of the inhibitors, and then media were subjected to immunoprecipitation.

ble EGF-receptor [11]. The studies described here suggest that exogenously added EGF may exert receptor regulation at yet another level - the level of post-translational maturation and export. With respect to the pathway of EGF action we considered the possibility of C-kinase, which is known to be involved in receptor regulation at pre-translational levels [12]. However this kinase appears to have no direct involvement in post-translational receptor expression as seen by the inability of TPA to modulate this process (data not

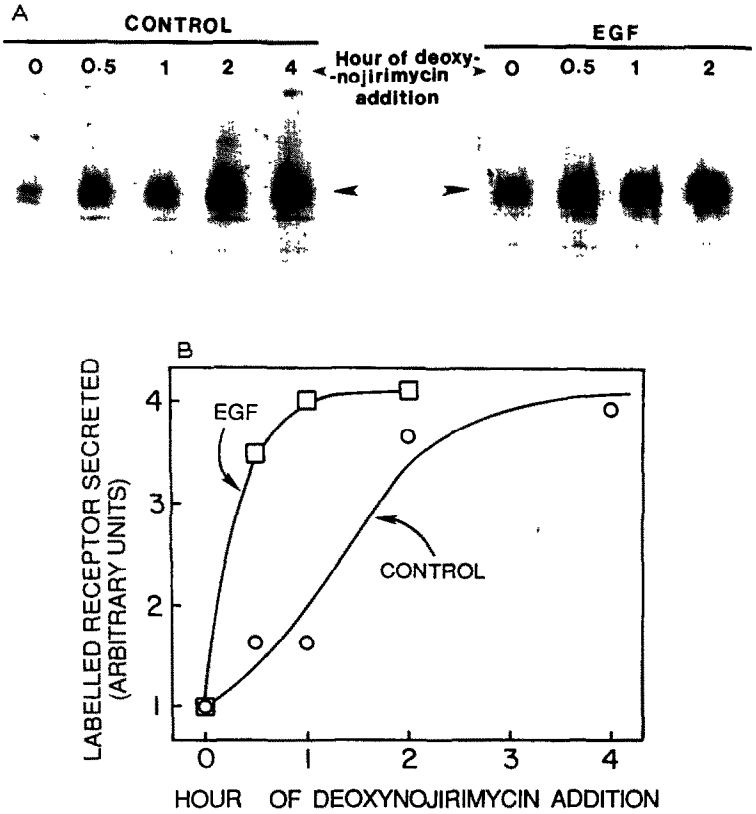

Fig. 4. EGF stimulates the deoxynojirimycin-sensitive processing event. Cells in 16 mm dishes were labelled with [^{35}S]methionine for 2 h, then chased at 37°C for 12 h in 0.3 ml of DME-0.1% BSA containing 20 nM EGF or no EGF. Deoxynojirimycin (4 mM) was added at the indicated time points of chase. All cellular media were collected at 12 h and subjected to immunoprecipitation. (A) Autoradiographic depiction of results; (B) plot of the densitometric data. The results are representative of three seperate experiments.

shown). On the other hand the lack of inhibition by cycloheximide indicates that the EGF effect requires no new protein synthesis. It remains to be seen what EGF-induced cellular signalling pathways are involved in this soluble receptor export system, and whether EGF also modulates the post-translational transport characteristics of the intact transmembrane EGF-receptor.

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