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Evidence for Intracellular Down-Regulation of the Epidermal Growth Factor (EGF) Receptor during Adenovirus Infection by an EGF-Independent Mechanism

PATRICIA HOFFMAN,[1] PREMEELA RAJAKUMAR,[2] BRIAN HOFFMAN,[1] RITA HEUERTZ,[2] WILLIAM S. M. WOLD,[2] AND CATHLEEN R. CARLIN[1]*

Department of Physiology and Biophysics, School of Medicine, Case Western Reserve University, 2119 Abington Road, Cleveland, Ohio 44106,[1] and Institute for Molecular Virology, St. Louis University Medical School, St. Louis, Missouri 63110[2]

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We have reported previously that human group C adenoviruses down-regulate the epidermal growth factor (EGF) receptor (EGF-R) (C. R. Carlin, A. E. Tollefson, H. A. Brady, B. L. Hoffman, and W. S. M. Wold, Cell 57:135–144, 1989). Expression of a 13.7-kDa protein encoded by a gene in the E3 transcription unit is necessary and sufficient for this effect (Carlin et al., Cell, 1989; B. L. Hoffman, A. Ullrich, W. S. M. Wold, and C. R. Carlin, Mol. Cell. Biol. 10:5521–5524, 1990). We show here that EGF-R down-regulation is accelerated in cells which overexpress the receptor when these cells are infected with virus mutants that overproduce the 13.7-kDa protein compared with wild-type virus. This is in contrast to EGF stimulation, for which others have shown that high concentrations of ligand are associated with low rates of receptor internalization in EGF-R-overexpressing cells (D. Kuppuswamy and L. J. Pike, J. Biol. Chem. 264:3357–3363, 1989; H. S. Wiley, J. Cell Biol. 107:801–810, 1988). We also show that the E3 protein is not present in media conditioned by infected cells and that it does not induce secretion of an EGF-like autocrine factor. Moreover, while mature membrane-bound EGF-R is down-regulated, the precursor of the membrane-bound form is not. Adenovirus infection also does not affect receptor-related molecules expressed in the secretory pathway. Interestingly, adenovirus-induced down-regulation is not regulated by concentrations of EGF associated with a slow rate of internalization in A431 cells. This suggests that 13.7-kDa protein expression triggers receptor entry by a novel ligand-independent pathway or, alternatively, that it compensates for a cellular factor that may be rate limiting during EGF-mediated endocytosis.

Adenoviruses cause a variety of illnesses in humans, chief among them acute viral respiratory disease (31). Adenoviruses also establish and sustain latent infections. Although relatively little is known about host-virus interactions, adenovirus gene products that regulate host-specific proteins have been identified. The adenovirus E3 protein E19, for example, prevents the transit of class I antigens of the major histocompatibility complex to the cell surface (21). This may help to counteract host immune responses, since reduced expression of class I antigens in vitro leads to reduced cytolysis of adenovirus-infected cells by class I antigen-specific cytotoxic T cells (5). Whether this activity has the same effect during primary infections versus periods of reactivation or contributes in any way to latency remains unknown.

The primary targets for infection by group C adenoviruses are epithelial cells lining the upper respiratory tract (34). It is therefore significant that the epidermal growth factor (EGF) receptor (EGF-R), a ligand-activated protein tyrosine kinase (PTK) (36), is rapidly down-regulated by adenovirus in cultured human cells. The viral gene responsible for this effect also lies in the E3 transcription unit and encodes a 91-amino-acid protein (8). Retrovirus-mediated gene transfer has shown that expression of this protein is sufficient to down-regulate EGF-R (15). The primary translation product in vivo has an apparent molecular mass of 13.7 kDa (16). A second species with an apparent molecular mass of 11.3 kDa and lacking the N-terminal 22 amino acids is derived rapidly by proteolysis (19). It is also known that the molecule is a disulfide-bonded dimer (16). Experimental evidence and computer modeling suggest that the 13.7-kDa protein (13.7 kDa) is an integral membrane protein with two membrane-spanning α helices and cytosolic N and C termini (15, 16, 19). Moreover, 18 amino acids near the 13.7-kDa C terminus show significant sequence homology with the juxtamembrane domain at the cytoplasmic face of EGF-R (8). The E3 protein is therefore quite different from viral proteins such as vaccinia virus growth factor (1) which resemble EGF and interact with the ligand-binding domain of EGF-R.

Cell lines that overexpress EGF-R, such as epithelial carcinoma-derived A431 cells, are unusual in that increasing concentrations of EGF decrease the rate of internalization of ligand-receptor complexes (20, 40). This has led to speculation that one or more elements in the endocytotic pathway are saturable. These cells also express both membrane-bound and secreted forms of EGF-R (35, 39). It was therefore of interest to determine the effect of infecting these cells with group C adenoviruses. We show here that in contrast to ligand-induced down-regulation, down-regulation of EGF-R is accelerated when the 13.7- and 11.3-kDa proteins (13.7 kDa/11.3 kDa) are overexpressed. The activity of 13.7 kDa/11.3 kDa is intracellular and confined to fully processed, membrane-bound forms of the receptor. Moreover, down-regulation of EGF-R by adenovirus is not subject to regulation by molecules that appear rate limiting during EGF stimulation. Taken together, these data support a model involving direct interaction between the viral proteins and

* Corresponding author.

EGF-R or indirect interaction via a cellular intermediate. Additionally, the mechanism employed by adenovirus either bypasses the saturable endocytotic element required for ligand-mediated internalization or provides a substitute so that this step is no longer rate limiting.

MATERIALS AND METHODS

Cells and viruses. Human epithelial carcinoma-derived A431 cells (13) were grown in Dulbecco's modified minimal essential medium supplemented with 2 mM glutamine and 10% fetal bovine serum, and HerC cl transfectants (28) were grown in Dulbecco's modified minimal essential medium-F12 medium containing 250 nM methotrexate, 2 mM glutamine, and 10% fetal bovine serum. Virus stocks were propagated in spinner cultures of human KB cells maintained with Joklik's modified minimal essential medium supplemented with 5% horse serum. The isolation and characterization of H5/2*rec*700, an adenovirus type 5-adenovirus type 2-adenovirus type 5 recombinant wild-type group C virus, and virus mutants are described elsewhere (2, 3). Briefly, three mutants overproduce the E3 message which encodes 13.7 kDa (i.e., message *f*) at the expense of the major E3 transcript seen in wild-type virus (i.e., message *a*): *in*723 and *in*724 are insertion mutants that increase the distance between the 3′ splice site of message *f* and the polyadenylation site for message *a*, and *pm*760 contains a point mutation that eliminates the polyadenylation signal for message *a*. *dl*752 and *dl*753 are deletion mutants that delete 14 and 207 nucleotides, respectively, from the 13.7 kDa open reading frame.

Cell labeling, immunoprecipitation, and immune complex assays. Cells were labeled with L-[^{35}S]cysteine (200 μCi/ml; >600 Ci/mmol; New England Nuclear, Wilmington, Del.) in cystine- and serum-free medium as described in the figure legends. Cell lysates were prepared with 1% (wt/wt) Nonidet P-40–0.5% (wt/wt) sodium deoxycholate–0.1% (wt/wt) sodium dodecyl sulfate (SDS) in 50 mM Tris (pH 8.0) supplemented with 150 mM NaCl–0.2 mM phenylmethylsulfonyl fluoride, leupeptin (0.5 μg/ml), and pepstatin (0.7 μg/ml) for viral protein analysis or with 1% (wt/wt) Nonidet P-40 in 0.1 M Tris (pH 6.8) supplemented with 15% (wt/wt) glycerol, 2 mM EDTA, 1 mM EGTA [ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid], and the protease inhibitors listed above for EGF-R analysis. Detergents and protease inhibitors were from Boehringer Mannheim Biochemicals (Indianapolis, Ind.). Radiolabeled culture supernatants were also conserved in some experiments.

Immunoprecipitations were carried out by using antibodies absorbed onto protein A-Sepharose CL-4B beads (Sigma Chemical Co., St. Louis, Mo.). Antibodies to 13.7 kDa were raised in rabbits by using a synthetic 15-mer corresponding to the C terminus coupled with keyhole limpet hemocyanin. The specificity of the peptide antibody has been documented previously (15, 33). Immunoprecipitation of EGF-R was done with a receptor-specific monoclonal antibody, EGF-R1 (37). Immune complex EGF-R autophosphorylation assays were carried out by the method of Carlin and Knowles (6). Extracts from equivalent numbers of cells were used when comparisons of cells infected with different viruses were to be made (see Fig. 2 and 4). Immune complexes were solubilized in Laemmli buffer and separated by SDS-polyacrylamide gel electrophoresis (22). Gels with L-[^{35}S]cysteine-labeled proteins were processed for fluorography by using En^{3}Hance (New England Nuclear) according to the manufacturer's instructions. Radioactivity in gel slices digested with Protosol (New England Nuclear) was quantitated by using a Beckman model LS-250 liquid scintillation counter.

^{125}I-EGF binding assays. Receptor-grade mouse EGF (Sigma) was radioiodinated by the chloramine-T method (specific activity, ~2 × 10^8 cpm/μg). Binding assays were performed at 24 h postinfection (p.i.) by using 5 ng of ^{125}I-EGF per ml (see reference 6 for further detail); nonspecific binding was measured by using 500 ng of EGF per ml. Cells were incubated with ligand for 20 min at 37°C and solubilized with 1 M NaOH. All determinations were made in duplicate. Radioactivity was measured with a Beckman model 310 gamma counter.

Northern (RNA) blot analysis. Preparation of cytoplasmic RNA and purification of poly(A)$^+$ RNA by oligo(dT) (Pharmacia, Piscataway, N.J.) chromatography were carried out according to standard protocols (24). Poly(A)$^+$ RNA was fractionated on 1% formaldehyde gels and transferred to Nytran (Schleicher & Schuell, Keene, N.H.) by capillary action. Blots were hybridized with a ^{32}P-labeled RNA probe designed to recognize all EGF-R transcripts expressed in A431 cells; this probe was prepared by subcloning the *Cla*I-*Eco*RI fragment containing nucleotides 1 to 457 of the human EGF-R cDNA pE15 (27) into pGEM-3 (Promega, Madison, Wis.). Hybridization was for 12 h at 65°C.

RESULTS

The extent to which EGF-R is down-regulated in cells that overexpress EGF-R is a function of the level of 13.7 kDa/11.3 kDa expression. EGF-R down-regulation in two cell lines that overexpress the receptor as a consequence of gene amplification was examined. In A431 cells, multiple gene copies are associated with hypotetraploidy for chromosome 7 (10, 30). HerC cl cells are NIH 3T3 transfectants containing human EGF-R cDNA driven by a heterologous promoter and coamplified with dihydrofolate reductase (28). We compared receptor abundance in these cells following infection with wild-type virus versus infection with virus mutants engineered to overproduce the E3 protein. Phenotypes of three of these mutants with regard to 13.7 kDa and 11.3 kDa expression are shown in Fig. 1. Since EGF-R is a PTK, EGF-R abundance can be measured by using immune complex assays for receptor-mediated autophosphorylation. Although there was a clear reduction in EGF-R PTK activity following infection with wild-type virus versus a 13.7 kDa/11.3 kDa deletion mutant by 24 h p.i., the effect was more pronounced after infection with mutants that overproduce these proteins (Fig. 2). Similar results were obtained when EGF-R cell surface expression was assessed by measuring the ability of A431 cells to bind ^{125}I-EGF (Fig. 3). In addition, time course experiments showed that down-regulation was accelerated by infection with virus mutants that overexpress 13.7 kDa/11.3 kDa. Whereas the receptor autophosphorylating capacity in cells infected with wild-type virus was down-regulated between 18 and 24 h p.i. (data not shown), loss of PTK activity in cells infected with virus mutants overexpressing 13.7 kDa/11.3 kDa was seen as early as 12 h p.i. (Fig. 4); EGF-R autophosphorylating activity in cells infected with a 13.7 kDa/11.3 kDa deletion mutant remained steady from 6 to 24 h (data not shown). Taken together, these results demonstrate that down-regulation by 13.7 kDa/11.3 kDa in cells that overexpress EGF-R is dose dependent.

EGF-R mRNA levels and de novo protein synthesis during adenovirus infection are not affected by 13.7 kDa/11.3 kDa

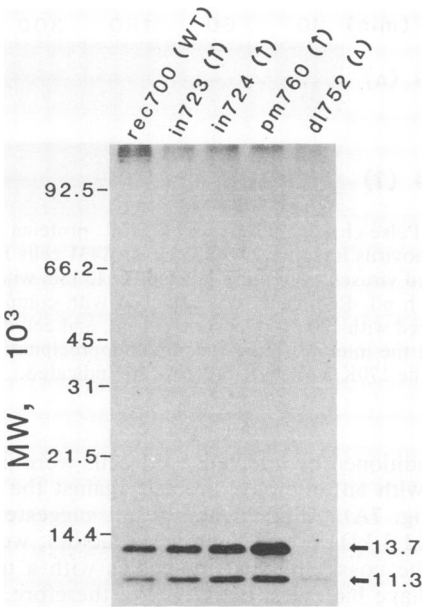

FIG. 1. Immunoprecipitation of 13.7 kDa and 11.3 kDa from A431 cells following infection with wild-type (WT) and mutant viruses. A431 cells were infected with 200 PFU per cell; phenotypes of virus mutants with regard to 13.7 kDa and 11.3 kDa expression are indicated in the figure. Cells were metabolically labeled with L-[^{35}S]cysteine from 12 to 16 h p.i., and cell extracts were immunoprecipitated with a rabbit antiserum specific for 13.7 kDa and 11.3 kDa. Proteins were resolved on a 15% polyacrylamide gel. *dl*752 has an in-frame deletion near the 5′ end of the coding sequence; although an aberrant protein is expressed at low levels, this mutant does not cause EGF-R down-regulation (8). No immunoprecipitable bands are found for cells infected with another 13.7 kDa/11.3 kDa deletion mutant used in this study, *dl*753 (33). Bands representing the 13.7 kDa species were excised, and counts per minute were as follows: *rec*700, 2,861; *in*723, 3,430; *in*724, 4,915; *pm*760, 9,583; background, 1,266. Molecular weight markers: phosphorylase *b*, 92,500; bovine serum albumin, 66,200; ovalbumin, 45,000; carbonic anhydrase, 31,000; soybean trypsin inhibitor, 21,500; lysozyme, 14,400.

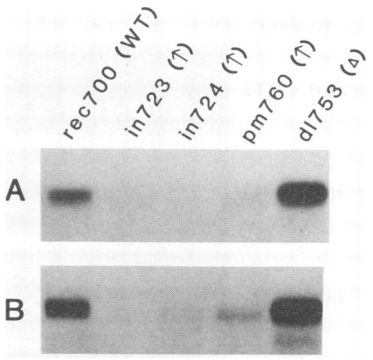

FIG. 2. EGF-R expression in A431 (A) and HerC cl (B) cells 24 h p.i. Cells were infected with 200 PFU per cell; phenotypes of virus mutants are indicated. Cell extracts were prepared at 24 h p.i., and extracts from equivalent cell numbers were immunoprecipitated with EGF-R1. EGF-Rs were analyzed for autophosphorylating activity in an immune complex. Proteins were resolved on a 7.5% acrylamide gel. WT, wild type.

expression. Previous studies (8) concerned the action of 13.7 kDa/11.3 kDa on EGF-R resident at the cell surface at the time cells are infected with adenovirus. The experiments described here address the fate of EGF-R molecules synthesized during infection. A431 cells, in addition to the full-length receptor, also produce a receptor variant protein encoded by a second gene which is shed into culture supernatant (35, 39); both receptor species are glycoproteins with N-linked oligosaccharides that are partially processed from high mannose to complex carbohydrate upon passage through the Golgi complex (7). Northern blot analysis showed that the overall level of receptor mRNAs expressed by these cells was not affected by expression of 13.7 kDa/11.3 kDa at 15 h p.i. (Fig. 5A). Similarly, when receptor proteins were immunoprecipitated from infected cells metabolically labeled with L-[^{35}S]cysteine, the levels of receptor biosynthetic precursors (i.e., 160,000-molecular-weight precursor [160K precursor] and 95K precursor) were unchanged by viral protein expression (Fig. 5B). There was, however, a clear reduction of fully processed mature receptor (i.e., 170K receptor) in cells expressing 13.7 kDa/11.3 kDa, suggesting that it is turning over more rapidly shortly after modification of N-linked oligosaccharide. The amount of the secreted form of the receptor shed into culture supernatant (i.e., 110K receptor), however, was not affected by adenovirus (Fig. 5C). The most likely explanation for this is that secreted EGF-R lacks sequences necessary for interaction with viral or intermediary proteins, although it may also be that viral and/or intermediary proteins are not expressed in the secretory pathway.

13.7 kDa/11.3 kDa expression does not cause increased turnover of EGF-R biosynthetic precursor. It has been previously hypothesized, on the basis of their ability to activate receptor precursor molecules in early biosynthetic compartments, that certain viral proteins utilize intracellular autocrine loops. For example, expression of v-*sis*, the transforming protein of simian sarcoma virus derived by transduction of the PDGF-B gene (38), causes accelerated turnover of the platelet-derived growth factor (PDGF) receptor biosynthetic precursor (17). To determine whether this was the case for adenovirus, A431 cells were pulse-labeled during infection to assess the stability of receptor precursor molecules. As shown in Fig. 6, the 160-kDa EGF-R precursor was detectable after a 30 or 60 min chase in cells infected with a virus mutant lacking 13.7 kDa/11.3 kDa; while trace amounts of

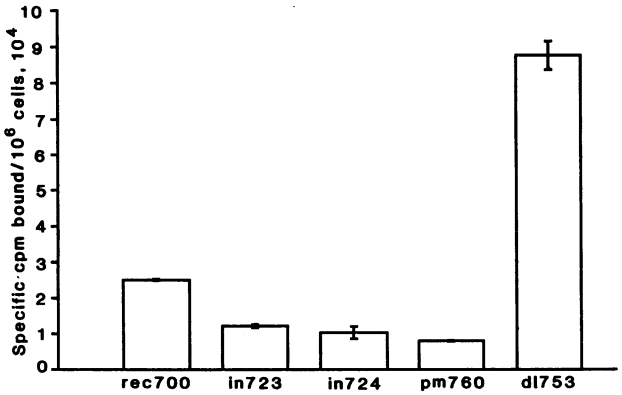

FIG. 3. Specific ^{125}I-EGF binding by A431 cells following infection with wild-type and mutant viruses. A431 cells were infected with the indicated viruses at 200 PFU per cell. Nonspecific binding ranged from 6% (*rec*700) to 15% (*pm*760). The error bars represent the sum of the standard deviations for total and nonspecific binding.

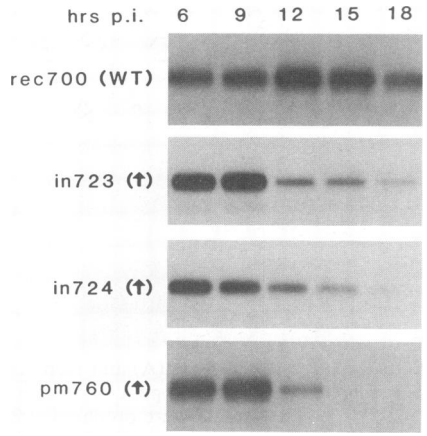

FIG. 4. Time course of EGF-R down-regulation in A431 cells following infection with wild-type (WT) and mutant viruses. A431 cells were infected with the indicated viruses at 200 PFU per cell. Cell extracts were prepared at the indicated intervals and analyzed for receptor autophosphorylating activity in an immune complex as described in the legend to Fig. 2.

precursor were seen at 3 h, the 170-kDa mature receptor was the predominant species. These results are identical to what is seen during EGF-R biosynthesis in uninfected cells (7). As expected, turnover of the mature receptor in cells infected with a virus mutant that overproduces 13.7 kDa/11.3 kDa was rapid. The expression of precursor receptor, however, was essentially the same as that observed for cells infected with the deletion mutant. Hence, in contrast to v-*sis*, expression of 13.7 kDa/11.3 kDa does not cause premature degradation of the biosynthetic EGF-R precursor.

13.7 kDa/11.3 kDa is not present in media conditioned by infected cells and does not induce secretion of an autocrine factor. Neither of the viral proteins could be detected when

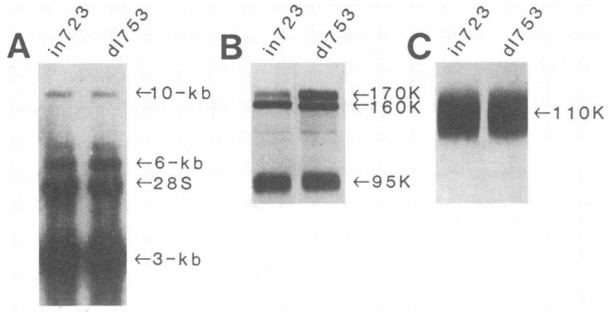

FIG. 5. EGF-R mRNA levels (A) and de novo protein synthesis (B and C) during adenovirus infection of A431 cells. A431 cells were infected with the indicated virus mutants at 200 PFU per cell. (A) Cytoplasmic RNA was prepared at 15 h p.i. Poly(A)$^+$ RNA (5 μg) was fractionated on a 1% formaldehyde gel. Hybridization was with the EGF-R-specific riboprobe described in Materials and Methods. The 6- and 10-kb species encode full-length receptor, and the 3-kb species encodes the truncated receptor-related species shed by A431 cells into culture supernatant. Residual 28S RNA hybridizes with the probe nonspecifically. (B and C) Cells were metabolically labeled with L-[^{35}S]cysteine from 15 to 19 h p.i. Cell extracts (B) and culture supernatants (C) were immunoprecipitated with EGF-R1. The 95K and 160K species are biosynthetic precursors of the 110K and 170K species, respectively (7); the 110K EGF-R-related protein is a truncated molecule expressed in A431 cells and shed into culture supernatant.

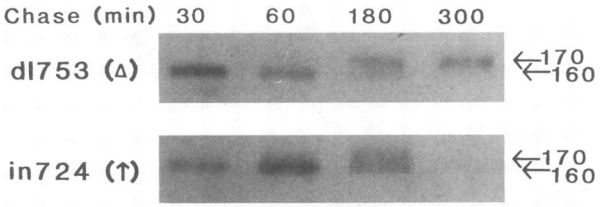

FIG. 6. Pulse-chase analysis of EGF-R proteins synthesized during adenovirus infection of A431 cells. A431 cells infected with the indicated viruses were pulse-labeled for 15 min with L-[^{35}S]cysteine at 15 h p.i. Radiolabel was replaced with complete medium supplemented with 120 μg of cystine per ml, and cell extracts were prepared at the intervals indicated. Immunoprecipitation was with EGF-R1. The 170K and 160K proteins are indicated.

media conditioned by infected A431 cells were immunoprecipitated with an antibody directed against the C-terminal 15-mer (Fig. 7A). Although this result suggested that 13.7 kDa and 11.3 kDa are not autocrine factors, we could not rule out the possibility that molecules with a truncated C terminus have biological activity. We therefore questioned whether a truncated viral protein or, alternatively, a protein induced by 13.7 kDa/11.3 kDa was secreted by treating uninfected cells with media conditioned by infected cells to look for evidence of EGF-R down-regulation. The purpose

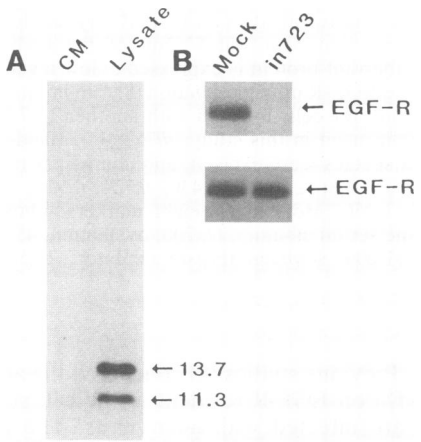

FIG. 7. Analysis of media conditioned by adenovirus-infected cells for 13.7 kDa and 11.3 kDa expression. (A) A431 cells were infected with *in*723 at 200 PFU per cell and metabolically labeled as described in the legend to Fig. 1. Conditioned medium (CM) and cell lysates were immunoprecipitated with a rabbit antiserum specific for 13.7 kDa and 11.3 kDa. Control immunoprecipitations of conditioned media using EGF-R1 to detect secreted EGF-R (Fig. 5C) were also carried out. Proteins were resolved on a 15% polyacrylamide gel. (B) Uninfected A431 cells that had been metabolically labeled with L-[^{35}S]cysteine for 1 h received the following treatments. (Top panel) Cells were mock infected (Mock) or infected with *in*723 at 200 PFU per cell; cell lysates were prepared at 24 h p.i. (Bottom panel) Radiolabeled medium was replaced with complete medium supplemented with 120 μg of cystine per ml, and cells were incubated for an additional 5 h. These cells were then refed with freshly harvested conditioned media from mock-infected cells or cells infected with *in*723; cell lysates were prepared 10 h later. Conditioned media were collected at 14 h p.i. on the basis of time course experiments showing that maximal down-regulation in cells infected with *in*723 occurs between 12 and 14 h p.i. (Fig. 4). Cell lysates were immunoprecipitated with EGF-R1, and proteins were analyzed on a 7.5% acrylamide gel.

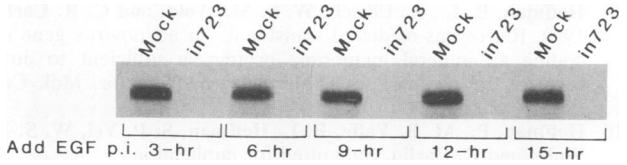

FIG. 8. EGF-R stability in A431 cells following infection with a virus mutant that overproduces 13.7 kDa/11.3 kDa. A431 cells were metabolically labeled with L-[^{35}S]cysteine for 1 h and then were either mock infected or infected with *in*723 at 200 PFU per cell. Cells were stimulated with 1.7×10^{-9} M EGF as indicated. Cell lysates prepared at 24 h p.i. were immunoprecipitated with EGF-R1.

of the assay used was to examine EGF-R stability by immunoprecipitating EGF-R from cells prelabeled with L-[^{35}S]cysteine before infection. When A431 cells were infected with a virus mutant that overproduces 13.7 kDa/11.3 kDa, EGF-R degradation was complete by 24 h p.i. (Fig. 7B, top panel). When uninfected cells were treated with supernatant conditioned by cells infected with this mutant, however, EGF-R stability was unaffected (Fig. 7B, bottom panel). These results suggest that EGF-R down-regulation during adenovirus infection is mediated intracellularly.

Comparison of EGF-R expression during EGF stimulation and adenovirus infection in A431 cells. We next examined the effect of treating A431 cells with EGF during adenovirus infection. These experiments were done by using the receptor stability assay described for experiments whose results are shown in Fig. 7B. In agreement with data from other laboratories, EGF-R turnover was essentially undetectable when uninfected A431 cells were stimulated with a concentration of EGF associated with a slow rate of internalization (i.e., 1.7×10^{-9} M; see reference 40) for periods up to 21 h (lanes receiving mock treatment in Fig. 8). Addition of the same concentration of EGF to infected cells, however, did not alter EGF-R turnover induced by 13.7 kDa/11.3 kDa (lanes *in*723 in Fig. 8). Hence, the cellular mechanism that causes lowered internalization rates in response to high concentrations of ligand does not affect down-regulation by adenovirus.

DISCUSSION

The evidence presented here shows that adenovirus employs a novel mechanism to down-regulate EGF-R. As shown in Fig. 9, EGF-R is routed to lysosomes from the cell surface during adenovirus infection. Unlike the situation with viral proteins that are biologically active autocrine growth factors, however, this occurs independent of secreted proteins that bind to the receptor extracellularly. Moreover, although it is possible that mature EGF-R is directed to lysosomes en route to the plasma membrane from a late biosynthetic compartment (i.e., *trans* Golgi), newly synthesized EGF-R precursor is not targeted for degradation. This contrasts with the intracellular autocrine loop mechanism utilized by v-*sis*, which causes premature transport of underglycosylated PDGF receptor to lysosomes from an early biosynthetic compartment (i.e., the endoplasmic reticulum or *cis* Golgi) (17). Intracellular EGF-R down-regulation in a membrane-associated compartment is consistent with our demonstration that 13.7 kDa and 11.3 kDa are integral membrane proteins (15, 19).

It is interesting that EGF-R down-regulation by 13.7 kDa is dose dependent, since this implicates a mechanism involving direct or indirect interactions between these two molecules. We have noted previously that there is sequence similarity between the C terminus of 13.7 kDa and the cytoplasmic juxtamembrane domain of EGF-R (8). It has been proposed previously that when cells are stimulated with EGF, EGF-Rs are stabilized in oligomeric complexes prior to internalization and degradation (29). If the sequence shared by 13.7 kDa and EGF-R is sufficient to mimic receptor intermolecular interactions, then the formation of hetero-oligomers between 13.7 kDa and EGF-R might account for adenovirus-mediated down-regulation. Efforts to test this hypothesis are under way in this laboratory.

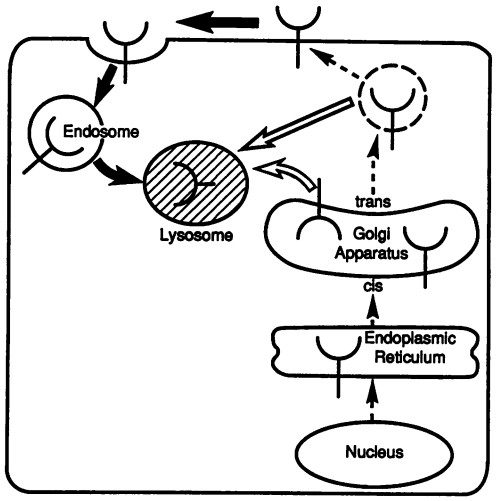

FIG. 9. Schematic showing proposed sites of EGF-R down-regulation during adenovirus infection. Solid arrows show putative routing of EGF-R internalized from the cell surface during adenovirus infection (8). Solid arrowheads with broken lines show intracellular routing of newly synthesized EGF-R. Open arrows show that EGF-R synthesized during adenovirus infection may be down-regulated from a late biosynthetic compartment as well as from the cell surface.

The physiological consequences of adenovirus-mediated EGF-R down-regulation remain speculative. Secreted molecules such as vaccinia virus growth factor probably stimulate EGF-like responses in infected cells as well as surrounding uninfected cells and ultimately increase virus yield, since virus production in vivo is attenuated by inactivation of the vaccinia virus growth factor gene (4). The site of action of 13.7 kDa/11.3 kDa, however, is intracellular; moreover, E3 is nonessential for viral replication both in cultured cells (18) and in vivo (14). An alternative hypothesis is that 13.7 kDa/11.3 kDa expression attenuates cellular responsiveness to EGF or transforming growth factor α, which binds to the same receptor as EGF (32) and is secreted by activated macrophages (23). Down-regulation of EGF-induced production of stromelysin (26), for example, may help to counteract host responses linked to regulatory cascades activated by serine proteases. The ability to block receptor-mediated vectorial EGF transport in polarized epithelial cells (12, 25) or EGF-induced maturation of lung cells (9) may also be an advantage to adenovirus. The latter strategy might camouflage the infected cell from host responses targeted to differentiated cells or, alternatively, engender a cell that is better suited to harboring latent virus. Expression of Epstein-Barr virus latent membrane protein in keratinocytes, for example, blocks terminal differentiation (11).

Adenovirus-mediated EGF-R down-regulation in A431

cells is not regulated by ligand. This is important, since it implies that 13.7 kDa/11.3 kDa expression either bypasses or compensates for saturable endocytotic elements. Whether physiological conditions exist under which normal cells might employ a similar mechanism remains to be seen. Future studies to understand the biological strategy associated with EGF-R down-regulation during adenovirus infection will also lead to greater appreciation of the pleiotropic nature of growth factor signalling pathways.

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