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Authors
Firestone, GL
John, NJ
Yamamoto, KR

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Glucocorticoid-regulated Glycoprotein Maturation in Wild-type and Mutant Rat Cell Lines

Gary L. Firestone,* Nancy J. John,* and Keith R. Yamamoto‡

*Department of Physiology-­Anatomy and Cancer Research Laboratory, University of California at Berkeley, Berkeley, California 94720; ‡Department of Biochemistry and Biophysics, University of California at San Francisco, San Francisco, California 94143

Abstract. Glucocorticoid hormones can regulate the posttranslational maturation of mouse mammary tumor virus (MTV) precursor polyproteins in M1.54, a stably infected rat hepatoma cell line. We have used complement-mediated cytolysis to recover variants of M1.54 that fail to express MTV cell surface glycoproteins in a hormone-regulated manner (Firestone, G. L., and K. R. Yamamoto, 1983, Mol. Cell. Biol., 3:149-160). One such clonal isolate, CR4, is similar to wild-type with respect to synthesis of MTV mRNAs, production of the MTV glycoprotein precursor (gPr74 env) and a glycosylated maturation product (gp51), and hormone-induced processing of two MTV phosphoproteins. In contrast, three viral cell surface glycoproteins (gp78, gp70, and gp32) and one extracellular species (gp70s), which derive from gPr74 env in glucocorticoid-treated wild-type cells, fail to appear in CR4. CR4 showed no apparent alterations in proliferation rate, cell shape, or expression of total functional mRNA and bulk glycoproteins. We conclude that the genetic lesion in CR4 defines a highly selective hormone-regulated glycoprotein maturation pathway that alters the fate of a restricted subset of precursor species.

POLYPEPTIDES destined to be membrane associated or secreted undergo a series of specific processing and compartmentalization reactions during and after translation (21, 44, 50, 52, 57, 62, 63). Cell biological and genetic data (1, 11, 17, 48, 51, 52, 59) suggest that shuttle vesicles selectively transport polypeptides from the rough endoplasmic reticulum into the Golgi stacks, through the Golgi cisternae and to their final intra-­or extracellular locations, apparently subjecting the polypeptides to an ordered array of modifications. For example, different Golgi cisternae appear to contain specific glycosyltransferases that modify glycoprotein substrates during passage through individual compartments (7, 8, 10, 21, 44). In addition, fatty acid modification of certain polypeptide backbones appears to occur at specific loci within the Golgi region (7, 9, 55). Different proteins with similar final destinations appear to use separate transport pathways (15, 52, 58), and certain pathways seem to include branch points that specify alternative processing fates (3, 14, 19, 31). Given the complexity, generality, and overall importance of these events, it would not be surprising if they were exploited for regulation of the relative levels of specific final products. Indeed, secretion of ACTH (19, 32) and parathormone (6) may be modulated by cAMP, although little is known of the mechanisms of these phenomena.

Glucocorticoids appear to regulate the posttranslational maturation of mouse mammary tumor virus (MTV) glycoproteins and phosphoproteins in M1.54, an MTV-infected rat hepatoma cell line containing 10 stably integrated proviruses (13, 14). For example, the predominant viral glycoprotein produced by control cultures is an extracellular 51,000 Mr species (gp51s), whereas hormone-treated cells produce two extracellular forms (gp51s and gp70s), and three cell surface species (gp78, gp70, and gp32). In addition, dexamethasone facilitates maturation of two new viral phosphoproteins (p24 and p35), derived by proteolytic cleavage from their common phosphorylated polypeptide (14, 24). Finally, the hormonal signal has a third effect on MTV gene expression, stimulating the rate of MTV RNA synthesis by increasing selectively the efficiency of MTV promoter utilization (5, 18, 38-40, 43, 46, 53, 60, 61, 64). Thus, glucocorticoids govern the absolute levels as well as the maturation of MTV proteins in M1.54. The present study, together with previous evidence (13), suggests that the three glucocorticoid-regulated pathways are genetically distinct.

The normal cellular targets for the hormone-controlled posttranslational processes have not been identified; however, the MTV polypeptides provide sensitive and perhaps general substrates for identifying and characterizing the regulated pathways. For example, we have selected and analyzed M1.54 variants defective in the maturation of certain MTV glycoprotein species in the presence of hormone. One such mutant line, CR4, expresses MTV RNA normally but fails to produce mature cell surface viral glycoproteins (13). In this report, we define molecular features of hormone-regulated glycoprotein maturation by comparing the expres-

Abbreviations used in this paper: endo H, endo β-N-acetylglucosaminidase H; MTV, mouse mammary tumor virus.

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sion and processing of MTV glycoproteins in the parent and mutant cell lines.

**Materials and Methods**

**Materials**

L-[35S]Methionine (1,000 Ci/mmol) and t-[2-3H]mannose (16 Ci/mmol) were obtained from Amersham Corp. (Arlington Heights, IL); [9,10-3H(N)]-palmitic acid was from New England Nuclear (Boston, MA); dexamethasone was from Sigma Chemical Co. (St. Louis, MO); and agarose derivatized with castor bean lectin (Rcricin)-120 was from P-L Biochemical, Inc. (Milwaukee, WI). All antisera (4) were generous gifts of J. T. Young and R. D. Cardiff (Department of Pathology, University of California, Davis); endo-B-N-acetylglucosaminidase H (endo H) was a gift of Regis Kelly (Department of Biochemistry and Biophysics, University of California, San Francisco). All other reagents were of highest available purity.

**Cells and Method of Culture**

M1.54 is a cloned line of MTV-infected rat hepatoma tissue culture (HTC) cells (47); selection of its complement-resistant derivative CR4 has been described previously (42). Cells were cultured in DMEM supplemented with 5% horse serum as described (44). Where indicated, dexamethasone was added to the culture medium to a final concentration of 1 µM. Cells were radiolabeled with [35S]methionine in methionine-deficient medium, with [2-3H]mannose in glucose/mannose-deficient medium, and with [9,10-3H]palmitic acid in normal medium, in each case supplemented with 1% dialyzed FCS.

**Pulse-Chase Experiments**

Monolayer cultures were precultured with 1 µM dexamethasone for 24 h and pulse-labeled for 20 min with 100 µCi [35S]methionine or 150 µCi [2-3H]mannose; the cultures were rinsed several times with PBS and then incubated in medium supplemented with 1% horse serum and either unlabeled methionine (1 mg/ml) or unlabeled mannose (1 mg/ml). Where noted, dexamethasone was present in the medium throughout both incubation periods. At the indicated times, the secreted fraction was harvested by centrifugation of the culture medium at 600 g for 3 min; the resulting supernate was frozen immediately in liquid nitrogen and stored at −20°C. Cell monolayers were washed twice in ice-cold PBS, released from the plates in 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, and centrifuged at 600 g for 3 min; cell pellets were frozen immediately in liquid nitrogen and stored at −20°C. Acid hydrolysis and subsequent thin layer chromatography of [2-3H]mannose-labeled glycoproteins revealed that the radioiod label remained within mannose.

**Steady-state Incorporation of Radiolabeled Material**

Cultures were incubated 12 h at 37°C in appropriate media with either 50 µCi [35S]methionine, 100 µCi [2-3H]mannose, or 500 µCi [9,10-3H(N)]-palmitic acid; cellular and extracellular fractions were harvested as described above. Hormone-treated cultures were precultured with dexamethasone for 24 h as well as during the radiolabeling period. Approximately 10-fold more extracts were immunoprecipitated from any uninduced extracts to compensate for the independent glucocorticoid stimulation in MTV RNA synthesis. Intact cells were iodinated at the cell surface as described previously (12).

**Immunoprecipitation of MTV Polypeptides**

Cells were homogenized at 107 per ml in solubilization buffer (1% Triton X-100, 0.5% deoxycholate, 5 mM EDTA, 250 mM NaCl, 25 mM Tris-HCl, pH 7.5) and centrifuged 15 min at 20,000 g; the supernatant was used for immunoprecipitation. Secreted fractions were brought to 1% Triton X-100, 0.5% deoxycholate, and 5 mM EDTA just before immunoprecipitation. Total radiolabeled polypeptides were analyzed by precipitating an aliquot (10 µl) of the soluble fraction with 10% TCA. MTV proteins were immunoprecipitated from detergent-solubilized extracts using formaldehyde-fixed *Staphylococcus aureus* (26) by a low background assay described previously (42).

**SDS Gel Electrophoresis and Autoradiography**

*Staphylococcus aureus* pellets were solubilized in 25 µl SDS gel sample buffer (30) and 0.5 M dithiothreitol (DTT) at 100°C for 2 min; after a 2-min Eppendorf centrifugation, the supernatants were electrophoresed in SDS polyacrylamide gels containing 9.5% acrylamide. Polypeptides were subsequently fixed and stained overnight with 0.4% Coomassie Brilliant Blue in 8% acetic acid, 25% isopropanol. Gels were destained in 10% acetic acid for 4 h, impregnated with ENHANCE (New England Nuclear) for 1 h, rinsed in water for 1 h, dried at 80°C, and analyzed by fluorography on Kodak RP Royal Xomat film in the presence of a DuPont Cronex "Lightning Plus" intensifying screen at −70°C.

**Endo H Digestion**

Immunoprecipitated MTV polypeptides were digested with endo H by modification of the method of Zilberstein et al. (65). After eluting the radiolabeled antigens from *S. aureus* in 30 µl of SDS gel sample buffer, 30 µl of 0.3 M sodium citrate, pH 5.5, was added in the presence or absence of 0.5 mg/ml endo H and incubated for 16 h at 37°C. Radiolabeled proteins were precipitated with 700 µl of acidified acetone (acetone 5 N HCl, 40:1) at 4°C for 10 min; precipitated proteins were harvested by centrifugation at 10,000 g for 10 min. SDS gel sample buffer and DTT were added to the pellets for electrophoresis.

**Cell Proliferation Assay**

M1.54 and CR4 cells were plated at an initial density of 104 cells per 2-cm2 well (Corning multi-well plate; Corning Glass Works, Corning, NY) and treated 24 h after plating with 1 µM dexamethasone. At various times through 6-d time course, triplicate samples of cultures were fixed in 70% ethanol, dried in vacuo at room temperature, and 0.4 ml of 10% 3,5-diaminobenzoic acid-dihydrochloride (Aldrich Chemical Co., Milwaukee, WI) was added to each well. After 1 h at 60°C, 2 ml of 1 N HCl was added and the DNA-mediated fluorescence (22) in each solution monitored at excitation and emission wavelengths of 405 and 465-540 nm, respectively, in a Model 112 Turner fluorimeter. Under these conditions, a reading of one fluorescence unit equals 104 cells.

**RNA Isolation and In Vitro Translation**

Total RNA was isolated from M1.54 and CR4 cells exposed to 1 µM dexamethasone, translated in vitro in a rabbit reticulocyte lysate (41), and [35S]methionine-labeled proteins were analyzed by two-dimensional gel electrophoresis (35) as described previously (13).

**Results**

**Analysis of Mutant CR4 Proliferation, Cell Shape, and Functional mRNA**

Using complement-mediated cytolysis, the CR4 mutant line was isolated on the basis of its inefficient expression of cell surface MTV polypeptides in the presence of dexamethasone (13). Conceivably, gross differences in cell growth rate or shape could give rise to this phenotype. Glucocorticoids have been shown to affect cell proliferation, in particular producing a nearly complete inhibition of cell growth in certain hepatoma lines (20). As shown in the graph in Fig. 1, M1.54 and CR4 each display doubling times of ~25 h in the absence of hormone and both show only a modest (18%) decrease in growth rate in the presence of dexamethasone. Similarly, light microscopic comparisons of CR4 and M1.54 cultures propagated 6 d in the presence or absence of hormone (Fig. 1; four images), revealed no systematic differences in cell shape; independent of hormone treatment, each cell line was epithelial-like and somewhat flattened with polygonal structures. Thus, in contrast to a previous report (23), we concluded that cell shape per se does not appear to account for the defective localization of cell surface MTV polypeptides in CR4.

To survey the expression of functional mRNA in M1.54 and CR4, total cellular RNA was isolated from hormone-induced cells, translated in vitro in a rabbit reticulocyte ly-
Figure 1. Proliferation and shape of dexamethasone-treated and untreated M1.54 and CR4 cells. (Left) M1.54 and CR4 cells were plated in the presence or absence of 1 μM dexamethasone and the DNA content was analyzed by a diaminobenzoic acid fluorescence assay through a 6-d time course. Using a standard curve, DNA-mediated fluorescence was monitored at the indicated times and converted to cell number per cm². (Right) At the 6-d time point, cells were examined by light microscopy. Bar, 50 μm.

Figure 2. Two-dimensional gel electrophoresis of in vitro translation products from M1.54 and CR4 cells. The cell-free translation system was programmed with 10 μg of total RNA isolated from M1.54 (top) or CR4 (bottom) that had been propagated in the presence of 1 μM dexamethasone. Samples (2 μl) were analyzed by twodimensional electrophoresis with 80% pH 5-8 and 20% pH 3.5-10 ampholines in the isoelectric focusing (IEF) dimension. The molecular mass standards are β-galactosidase (120,000 Mᵋ), BSA (68,000 Mᵋ), ovalbumin (43,000 Mᵋ), aldolase (40,000 Mᵋ), and chymotrypsinogen (25,000 Mᵋ). The arrows denote the 42,000 Mᵋ (50-kD secreted glycoprotein) and 22,000 Mᵋ (α₁-acid glycoprotein) in vitro translation products induced by dexamethasone.
Expression of MTV Glycoproteins in M1.54 and CR4

To examine MTV glycoprotein maturation, M1.54 and CR4 cultures exposed to 1 μM dexamethasone for 24 h were pulse-labeled with [2-3H]mannose followed by additional incubation in the presence of excess unlabeled mannose. At various times, [2-3H]mannose-labeled viral glycoproteins were immunoprecipitated with anti-MTV antibodies, fractionated in SDS polyacrylamide gels, and visualized by fluorography. As shown in Fig. 3 (lanes E-H), dexamethasone-treated M1.54 initially synthesizes a 74,000 M₉ precursor glycoprotein (designated gPr74ₑ⁻ الحوار) that is subsequently matured into four major species (gP78, gp70, gp51, and gp32); several other minor or transiently expressed viral glycoproteins are also produced. Hormone-treated CR4 also produces gPr74ₑ⁻ الحوار, (upper panel, lane I), but its subsequent processing is dramatically different (lanes J-L); gp78, gp70, and gp32 expression is reduced or undetectable in CR4, whereas several other gPr74ₑ⁻ الحوار-derived species, most notably gp51, are produced competently. We have suggested previously that specific portions of the MTV protein maturation pathways are dexamethasone inducible. To compare the hormone-regulated species in M1.54 with those deficient in CR4, uninduced cultures of M1.54 were pulse-labeled with [2-3H]mannose, followed by subsequent incubation with excess unlabeled mannose, immunoprecipitation of MTV glycoproteins, and fractionation by electrophoresis. As shown in Fig. 3 (lanes A-D), untreated cells synthesize gP74ₑ⁻ الحوار but produce little detectable [2-3H]mannose-labeled gp78, gp70, and gp32. Thus, the production of gP74ₑ⁻ الحوار-derived glycoproteins in control M1.54 (lanes A-D) is strikingly similar to that in hormone-treated CR4 (lanes I-L), suggesting that the genetic lesion in CR4 specifically blocks the hormonal regulation of glycoprotein maturation.

This notion was tested further by analyzing the extracellular viral glycoproteins. We established previously that at steady state, hormone-treated M1.54 produce two free extracellular MTV polypeptides (70,000 M₉ and 51,000 M₉) in approximately equimolar amounts, while control cells secrete predominantly (>95%) the 51,000 M₉ species (14). Fig. 3 shows glycoproteins pulse-labeled with [2-3H]mannose.
nose that are secreted after a 120-min chase; dexamethasone-treated cells secrete equal levels of [2-\(^3\)H]mannose-labeled 70,000 \(M_r\) and 51,000 \(M_r\) species (lanes e-\(l\)) while control cultures secrete predominantly the 51,000 \(M_r\) product (lanes a-\(d\)); these extracellular viral glycoproteins, designated gp70s and gp51s, account for at least 60% of the total synthesized MTV glycoproteins at steady state. Importantly, hormone-treated CR4 secretes a drastically reduced level of gp70s relative to that released from M1.54 (lanes m-t). Thus, CR4 appears to carry a defect in a hormone-induced glycoprotein maturation pathway that controls the appearance of particular cellular and secreted viral glycoproteins.

Identification of Cell Surface MTV Glycoproteins

The expression in CR4 of a specific subset of gPr74-derived maturation products suggests that the CR4 mutation may uncouple two distinct pathways for MTV glycoprotein maturation, one of which yields cellular gp78, gp70, and gp32 in the presence of hormone. To examine the relationships of these hormone-regulated glycoproteins and to determine those species located at the cell surface, plasma membrane proteins accessible on intact cells were labeled by lactoperoxidase-catalyzed \([\(^{25}\)I]iodination (Fig. 4, lanes A-E), while a parallel culture was metabolically labeled with [2-\(^3\)H]mannose (Fig. 4, lanes F-J). Immunoprecipitation with anti-MTV antibodies (lanes A and F), monospecific anti-gp51 antibodies (lanes B and G), monospecific anti-gp32 antibodies (lanes C and H), monospecific anti-p28 antibodies (lanes D and H), or preimmune serum (lanes E and J). Immunoprecipitated polypeptides were fractionated by SDS PAGE and visualized by autoradiography or fluorography.

**Figure 4.** Cell surface-associated and intracellular MTV glycoproteins. Hormone-treated (24 h) M1.54 cells were either radiolabeled at the cell surface by lactoperoxidase-mediated \([\(^{25}\)I]iodination (lanes A-E) or metabolically labeled for 24 h with 300 \(\mu\)Ci [2-\(^3\)H]mannose (lanes F-J). Cell extracts were immunoprecipitated with anti-MTV antibodies (lanes A and F), monospecific anti-gp51 antibodies (lanes B and G), monospecific anti-gp32 antibodies (lanes C and H), monospecific anti-p28 antibodies (lanes D and H), or preimmune serum (lanes E and J). Immunoprecipitated polypeptides were fractionated by SDS PAGE and visualized by autoradiography or fluorography.
terminal portion of the polyprotein while gp32 is the carboxy-terminal domain and contains the transmembrane hydrophobic region (45). Interestingly, gp51 is associated with the cell surface despite its lack of a membrane anchor; conceivably gp51 may be associated with another primary integral membrane protein during transport to the cell surface. Consistent with this mechanism, we have shown that after multiple freeze-thaw at −100°C of isolated microsomes, which elutes luminal components of vesicles, gp51 remains associated with the membrane fraction (Haffar, O.K., C.P. Edwards, and G.L. Firestone, manuscript submitted for publication). Finally, the cell surface viral glycoproteins that fail to appear in CR4 (gp78, gp70, and gp32) have in common the polypeptide backbone for gp32 suggesting a crucial maturation or sorting domain may be contained within its structure. Thus, defective posttranslational maturation of the MTV glycosylated precursor appears to account for the absence of gp78, gp70, and gp32 from the CR4 cell surface. Since M1.54 and its derivatives contain 10 nonrandomly integrated MTV proviruses (13), all of which seem transcriptionally active (Harris, C., and K.R. Yamamoto, unpublished data), the CR4 defect is likely to reflect a lesion in a cellular gene, rather than a mutation in the env gene itself or elsewhere within MTV sequences.

Endo H Sensitivity of MTV Glycoproteins

In general, glycoprotein modification and maturation include addition of “high mannose” moieties in the rough endoplasmic reticulum which are further processed during transit through the Golgi stacks into “complex” oligosaccharide sidechains (21, 27, 28). Endo H, which specifically hydrolyzes the high-mannose species, was used to examine oligosaccharide maturation of [35S]methionine-labeled MTV glycoproteins from dexamethasone-treated cells. As shown in Fig. 5, both externalized species, gp51s and gp70s (whose expression in CR4 is reduced several fold), appear to contain a heterogeneous mixture of endo H-resistant and -sensitive carbohydrate side chains. Moreover, the pattern of endo H sensitivity is indistinguishable in M1.54 and CR4, implying that the altered maturation of particular MTV glycoproteins in CR4 is not due simply to a global defect in oligosaccharide processing.

Endo H treatment of [35S]methionine-labeled MTV glycoproteins immunoprecipitated from both M1.54 and CR4 yielded a 62,000 Mₐ, gPr74env derivative; a parallel experiment with [2-3H]mannose-labeled glycoproteins revealed that all of the [2-3H]mannose in gPr74env is removed (data not shown). In contrast, the [2-3H]mannose radiolabel in the membrane-associated gp78, gp70, gp51, and gp32 products remained attached, as expected for complex oligosaccharide side chains (data not shown). Finally, the glycosylated MTV precursors produced in M1.54 and CR4 migrate indistinguishably in two-dimensional gels (data not shown), suggesting that they are identical.

Attachment of [3H]Palmitate to MTV Glycoproteins

For certain plasma membrane proteins, covalently attached fatty acid residues appear to “anchor” the protein within the membrane (55); in the case of the vesicular stomatitis virus G protein, this lipid modification likely occurs during transport through the cis Golgi region soon after the acquisition of endo H resistance (7). When M1.54 and CR4 cultures were incubated for 24 h with [3H]palmitate in the presence or absence of dexamethasone, only the hormone-inducible gp78, gp70, and gp32 species became labeled (Fig. 6, right panel, lane C); in particular, no palmitate-labeled viral proteins were detected in either control (lane E) or hormone-treated (lane G) CR4. Labeling of parallel cultures with [35S]methionine (Fig. 6, left panel), showed that CR4 failed to express the polypeptide backbones for gp78, gp70, and gp32, rather than producing aberrant species unable to acquire the lipid modification.

Figure 5. Endo H sensitivity of extracellular viral glycoproteins. M1.54 (lanes C and D) and CR4 (lanes A and B) cells were incubated for 24 h with 1 μM dexamethasone in the presence of 150 μCi [35S]methionine. Equal amounts of viral glycoproteins were immunoprecipitated from extracellular fractions and subjected to endo H digestion as described in the text. MTV polypeptides were subsequently fractionated in SDS polyacrylamide gels and visualized by fluorography. Molecular mass standards were as described in Fig. 2. In a parallel experiment, endo H digestion of gPr74env resulted in a 62,000 Mₐ, species missing only its “high-mannose” oligosaccharides.
These results revealed that palmitate modification is a biochemical feature common to the cell surface MTV glycoproteins not expressed in CR4 cells. It is clear, however, that palmitate modification itself is neither hormone-dependent in M1.54 nor defective in CR4, since the specific activity of [3H]palmitate incorporated into TCA-precipitable material from dexamethasone-treated and untreated M1.54 and CR4 are similar (Table I). Table I also shows that [3H]fucose and [2-3H]mannose incorporation levels in the two lines are equivalent; moreover, hormone-treated and control M1.54 and CR4 produce similar [3H]palmitate-modified and [2-3H]mannose-labeled cellular glycoproteins (Haffar, O. K., and G. L. Firestone, unpublished data), establishing further that bulk glycoprotein expression in CR4 is unaffected.

**Discussion**

A variety of sorting mechanisms operate upon glycoproteins to effect their maturation and transit to membrane-associated or extracellular locations (17, 21). For example, in active or carrier-mediated pathways, vesicle or organelle-associated “sorting receptors” have been postulated to bind and segre-

Table 1. Incorporation of Lipid and Sugar Precursors by Wild-type and Mutant Cell Lines

| Cell line | Dexamethasone | [3H]Palmitate (cpm/mg) | [2-3H]Mannose (cpm/mg) | [3H]Fucose (cpm/mg) |
|-----------|---------------|------------------------|------------------------|---------------------|
| M1.54     | -             | 461,000                | 138,000                | 71,000              |
| M1.54     | +             | 395,000                | 135,000                | 68,000              |
| CR4       | -             | 421,000                | 129,000                | 75,000              |
| CR4       | +             | 454,000                | 145,000                | 76,000              |

* Cells were incubated with or without 1 μM dexamethasone for 24 h in the presence of 1 mCi [3H]palmitate, detergent solubilized, and the level of radioactive lipid incorporated into protein was determined by precipitation with 10% TCA. Specific incorporation is expressed as cpm/mg of precipitated protein.

* The incubation and assay conditions were the same as described in previous footnote, except that the cells were incubated with 200 μCi of [2-3H]mannose.

* The incubation and assay conditions were the same as described in the first footnote, except that the cells were incubated with 200 μCi of [3H]fucose.
gate selectively the appropriate glycoprotein substrates (2, 25), presumably by recognizing features of the polypeptide backbone or of the oligosaccharide side chains, as with the mannose-6-phosphate receptors involved in targeting lysosomal hydrolases (10, 56). Previous work of others suggests that some of these processes may be amenable to genetic dissection in cultured cells (57, 59). For example, Kabat and coworkers have isolated mutants defective in processing Friend murine leukemia virus or Friend spleen focus-forming virus envelope glycoproteins (16, 49), and Zilberstein et al. (65) have recovered variants that fail to produce cell surface-associated and lipid-modified vesicular stomatitis virus gene products. The novel feature of our results is the identification of a defect in a hormone-regulated posttranslational process. We conclude that the MTV glycoprotein precursor can be partitioned differentially into either a glucocorticoid-inducible or a constitutive trafficking pathway in cultured rat hepatoma cells. This finding demonstrates that global physiological regulators such as steroid hormones can effect acute changes in the products expressed from specific genes even after their transcription.

The ability of hormone-treated CR4 and control cultures of M1.54 to survive in the presence of MTV-specific antisem and serum reflects the absence of high levels of cell surface-associated gp78, gp70, and gp32; only in hormone-treated M1.54 does gPr74 env proceed efficiently through the maturation and sorting pathway that results in the membrane localization of these species. Thus, dexamethasone appears either to mediate an increase in a particular subset of transport or targeting activities or to stimulate the entry of gPr74 env into a pre-existing sorting route. It may be relevant in this regard that the cell surface glycoproteins not expressed in CR4 contain the polypeptide backbone for gp32 (the COOH-terminal domain of gPr74 env), perhaps indicating that a sorting signal resides within that structure.

In any case, the genetic lesion in CR4 displays a highly restricted phenotype, blocking selectively the hormonal induction of a glycoprotein maturation pathway that operates upon a specific subset of target precursors. Notably, the glucocorticoid receptor appears normal in CR4, and we have demonstrated that several hormone-responsive genes are regulated competently (13). Moreover, the gPr74 env species produced in hormone-treated and control M1.54 and CR4 are indistinguishable with respect to molecular weight, migration in two-dimensional gels, glycosidase sensitivity, and lectin affinity. In addition, bulk glycoprotein processing and compartmentalization appear normal in CR4, in contrast, for example, to the global alterations in intracellular protein traffic seen in the yeast secretory mutants defined by Schechman and coworkers (33, 34, 54). Similarly, control and hormone-treated M1.54 and CR4 are indistinguishable in the production of total palmitate-derivatized polypeptides (data not shown). Since the membrane-destined MTV glycoproteins normally produced in hormone-treated M1.54 and absent in CR4 are all palmitate modified, it seems likely that the hormone-regulated branch point resides at or before palmitate addition.

The regulatory mechanism by which the glucocorticoid receptor alters glycoprotein processing and compartmentalization is unknown. The putative hormone-regulated factors identified by the CR4 mutation might function directly in the processing of specific glycoproteins, or may operate as intracellular sorting receptors (25), analogous to the actions of egasyn and β2-microglobulin, which are essential for membrane association of β-glucuronidase (37) and HLA antigens (29, 36), respectively. Alternatively, the regulated factors might facilitate membrane vesicle flow into particular intracellular compartments, or maintain localization of specific target proteins subsequent to vesicle fusion (10, 25). In any case, inhibitors of RNA synthesis prevent the hormone-regulated glycoprotein maturation events (Haffar, O. K., and G. L. Firestone, manuscript submitted for publication). Thus, one simple interpretation is that glucocorticoids may regulate the transcription of genes encoding certain receptors or processing enzymes, and that the expression of one or more of these genes is defective in CR4.

Using MTV glycoproteins as targets for genetic selection and as intracellular biochemical probes, we have been able to detect and characterize a regulated posttranslational glycoprotein expression pathway without indications a priori of its existence, its normal cellular target proteins, or its biological significance. Conceivably, a pathway of this type could efficiently redirect the maturation and targeting fates of even large existing pools of glycoproteins simply by altering the synthesis or degradation of a single factor. This could facilitate subtle or dramatic transitions in specific glycoprotein localization, such as might be involved, for example, in cell membrane remodeling during development and differentiation. Of course, the precise nature of the regulated events and the regulatory signals themselves are likely to be cell specific. Thus, cAMP appears to control the externalization of mature ACTH in pituitary cells (19, 32). Through further characterization of CR4 and other genetic variants, it should be possible to identify cellular proteins whose maturation and targeting are controlled by glucocorticoids and to elucidate the normal biological function of this phenomenon.

This report is dedicated to the memory of Edward C. Heath, who supervised G. L. Firestone's doctoral research from 1975–1980 and whose guidance and friendship will always be remembered.

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