blocked maps to an approximately 3.3-kb Hind III fragment (Fig. 3A). It is interesting that DNase I hypersensitive site IV maps to this same region of the first intron. Site IV is the location of the major quantitative difference in DNase hypersensitivity between the 70Z/3B and the A20.2J cell lines, and may be functionally associated with the block to transcription elongation. Thus, it will be of interest to determine whether the DNase I hypersensitivity in this region reflects binding of a trans-acting factor that can mediate a block to transcription elongation.

REFERENCES AND NOTES

1. W. J. Boyle, M. A. Lampert, J. S. Lipsick, M. A. Baluda, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 4265 (1984); K. Klempnauer, J. Symonds, G. I. Evan, J. M. Bishop, *Cell* **37**, 537 (1984); K. Moelling *et al.*, *ibid.* **40**, 983 (1985).
2. M. Nunn, H. Weiher, P. Bullock, P. Duesberg, *Virology* **139**, 330 (1984); T. Graf, A. Von Kirchbach, H. Beug, *Exp. Cell Res.* **131**, 331 (1981); K. E. Rushlow *et al.*, *Science* **216**, 1421 (1982); K. Klempnauer, T. J. Gonda, J. M. Bishop, *Cell* **31**, 453 (1982); M. G. Moscovici *et al.*, *Virology* **129**, 65 (1983).
3. T. J. Gonda, D. K. Sheiness, J. M. Bishop, *Mol. Cell. Biol.* **2**, 617 (1982); E. H. Westin *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 2194 (1982); J. Coll *et al.*, *Exp. Cell Res.* **149**, 151 (1983); M. I. Mally, M. Vogt, S. E. Swift, M. Haas, *Virology* **144**, 115 (1985); S. P. Duprey and D. Boettiger, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 6937 (1985).
4. T. J. Gonda and D. Metcalf, *Nature (London)* **310**, 249 (1984); R. W. Craig and A. Bloch, *Cancer Res.* **44**, 442 (1984); I. R. Kirsch, V. Bertness, J. Silver, G. F. Hollis, *J. Cell. Biochem.* **32**, 11 (1986).
5. T. P. Bender and W. M. Kuehl, *Curr. Top. Microbiol. Immunol.* **132**, 153 (1986).
6. S. Lavu and E. P. Reddy, *Nucleic Acids Res.* **14**, 5309 (1986).
7. D. B. Sittman, R. A. Graves, W. F. Marzluff, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 1849 (1983).
8. U. Siebenlist *et al.*, *Mol. Cell. Biol.* **6**, 3042 (1986); C. Wu, *Nature (London)* **286**, 854 (1980).
9. T. P. Bender and W. M. Kuehl, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 3204 (1986).
10. M. Groudine and C. Casimir, *Nucleic Acids Res.* **12**, 1427 (1984).
11. R. G. Roeder, in *RNA Polymerase*, R. Losick and M. Chamberlin, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1976), p. 285.
12. D. L. Bentley and M. Groudine, *Nature (London)* **321**, 702 (1986).
13. A. Nepvue and K. B. Marcu, *EMBO J.* **5**, 2859 (1986).
14. D. Eick and G. W. Bornkamm, *Nucleic Acids Res.* **14**, 8331 (1986).
15. K. Kelly, B. H. Cochran, C. D. Stiles, P. Leder, *Cell* **35**, 603 (1983); J. B. Stern and K. A. Smith, *Science* **233**, 203 (1986).
16. J. Chirgwin, A. Aeybyle, R. McDonald, W. Rutter, *Biochemistry* **18**, 5294 (1979).
17. H. Lehrach, D. Diamond, J. M. Wozney, H. Boedtker, *ibid.* **16**, 4743 (1977).
18. P. W. J. Rigby *et al.*, *J. Mol. Biol.* **113**, 237 (1977).
19. P. A. Krieg and D. A. Melton, *Nature (London)* **308**, 203 (1984).
20. M. Groudine, M. Peretz, H. Weintraub, *Mol. Cell. Biol.* **1**, 281 (1981).
21. M. Linial, N. Gunderson, M. Groudine, *Science* **230**, 1126 (1985).
22. A. Dugaiczyk *et al.*, *Biochemistry* **22**, 1605 (1983).
23. C. B. Thompson and P. E. Neiman, *Cell* **48**, 369 (1987).
24. E. M. Southern, *J. Mol. Biol.* **98**, 503 (1975).
25. G. N. Wilson, B. A. Hollar, J. R. Waterson, R. D. Schmickel, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 5367 (1978).

26. We thank M. Groudine for advice and critical review of our data, G. Krystal and J. Battey for assistance ith the nuclear run-on assays and review of the manuscript, and G. Parris for help in preparing the manuscript. The opinions and assertions presented in this manuscript are solely those of the authors and are not to be construed as officially representing those of the Department of Defense or the Department of the Navy.

26 May 1987; accepted 7 August 1987

Expression of an Exogenous Growth Hormone Gene by Transplantable Human Epidermal Cells

JEFFREY R. MORGAN, YANN BARRANDON, HOWARD GREEN, RICHARD C. MULLIGAN

Retrovirus-mediated gene transfer was used to introduce a recombinant human growth hormone gene into cultured human keratinocytes. The transduced keratinocytes secreted biologically active growth hormone into the culture medium. When grafted as an epithelial sheet onto athymic mice, these cultured keratinocytes reconstituted an epidermis that was similar in appearance to that resulting from normal cells, but from which human growth hormone could be extracted. Transduced epidermal cells may prove to be a general vehicle for the delivery of gene products by means of grafting.

THE EPIDERMIS IS A STRATIFIED EPITHELIUM whose principal cell type, the keratinocyte, can be serially propagated in culture (*1*). Human keratinocytes grow rapidly under appropriate culture conditions, and it is possible to expand a 1-cm^2 biopsy to 1 m^2 of epithelium within about 3 weeks. The cultured epithelium can be detached from the surface of a dish as an intact sheet and grafted onto a suitable bed. Epithelia prepared in this way have been extensively used to regenerate an epidermis on burned humans (*2*). The cultivability of keratinocytes makes them suitable target cells for genetic manipulation. As a first step, we have used highly transmissible retroviral vectors to transfer DNA sequences into cultured keratinocytes. We now report successful transfer and expression of the gene encoding human growth hormone (hGH).

Gene transfer into keratinocytes was accomplished with transmissible retroviruses generated from the ψAM cell line (*3*). Recombinant viruses produced by the ψAM cell are free of detectable replication-competent virus and have an amphotropic host range and therefore can infect cells of various mammalian species, including the human.

The structures of the recombinant genomes that we used are shown in Fig. 1A. The vectors ZipneoSV(X) and DOL have been described (*4*). Cell lines derived from ψAM that produce ZipneoSV(X) virus (termed ψAM2275) and DOL-hGH virus (ψAM DOL-hGH) both yielded titers of 10^5 G418-resistant colonies per milliliter when assayed on NIH 3T3 cells. To infect human keratinocytes, we cocultivated them with lethally irradiated producer ψAM cells, since direct contact of virus-producing cells with recipient cells is an efficient means of transmitting the virus (*5*). The producer ψAM cells also provided fibroblast support necessary for optimal growth of the human keratinocytes (*1*). Three strains of keratinocytes derived from the foreskins of newborns (AY, YF17, and YF19) were cocultivated with lethally irradiated ψAM2275 or ψAM DOL-hGH cells for 3 to 4 days. The ψAM cells were selectively removed by a brief EDTA treatment, and the adherent keratinocytes were then detached with trypsin and inoculated onto a feeder layer of G418-resistant 3T3-J2 cells in medium containing G418 at 0.6 mg/ml. By 6 days, all drug-sensitive cells were eliminated, and the cultures were then grown in nonselective medium for 2 to 3 days before transfer.

The effectiveness of the gene transfer was determined by plating freshly infected keratinocytes at clonal density in medium with and without G418. The number of resistant colonies was found to be 0.1 to 0.5% of the total number of colonies, for both of the viral constructs and for the three strains of keratinocytes.

The G418-resistant keratinocyte colonies were indistinguishable from the uninfected colonies by their doubling time and morphological appearance, but their life span in culture was variably shorter.

The ZipneoSV(X) and DOL-hGH

J. R. Morgan and R. C. Mulligan, Whitehead Institute for Biomedical Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

Y. Barrandon and H. Green, Department of Physiology and Biophysics, Harvard Medical School, Boston, MA 02115.

recombinant retroviral genomes were detected in the cellular DNA from transduced keratinocytes by Southern blot hybridization (Fig. 1B). High molecular weight DNA prepared from the ψAM-producer cell lines and the G418-resistant keratinocytes was cleaved with Xba I and transferred to a nitrocellulose filter after fractionation by gel electrophoresis. Since Xba I cleaves once in the long terminal repeats (LTRs) of both the ZipneoSV(X) and DOL-hGH recombinant genomes, it excises unit-length proviral DNAs with expected sizes of 4.0 and 4.5 kb, respectively. Hybridization with a ^{32}P-labeled *neo* gene (neomycin phosphotransferase) sequence revealed the presence of the ZipneoSV(X) and DOL-hGH proviral genomes in the DNAs isolated from the corresponding ψAM-producer lines and strains of infected keratinocytes. Moreover, both ψAM cell lines transmitted recombinant proviruses of correct size to keratinocytes. ψAM DOL-hGH has a single proviral-sized *neo* fragment; this producer line was made by the infection of the ψAM cell line with an ecotropic DOL-hGH retrovirus derived from the ψ2 cell line (*6*). ψAM2275 has multiple *neo*-hybridizing fragments of which most are defective copies of the pZipneoSV(X) plasmid as a result of calcium phosphate–mediated transfection.

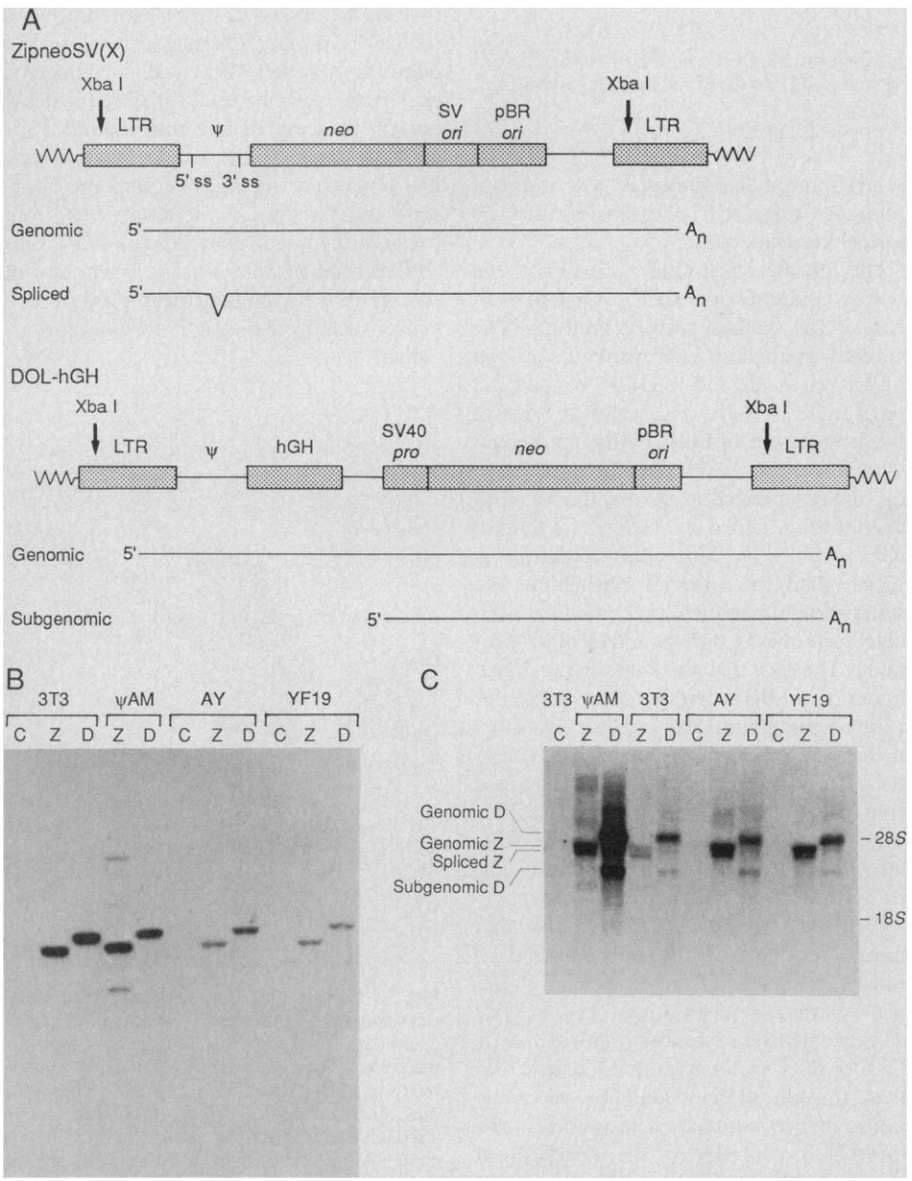

Fig. 1. Integration and transcription of recombinant retroviruses. (**A**) Proviral structures of pZipneoSV(X) and pDOL-hGH and the expected RNA transcripts are diagrammed. (**B**) Southern blot of cellular DNA (10 μg) digested with Xba I was probed with ^{32}P-labeled *neo* genes sequences labeled to high specific activity with [α^{32}P]deoxycytidine 5′-triphosphate by the random primer method (*14*). Each lane is labeled to indicate the cells from which the DNA was isolated. In the second and third lanes, 10 pg of plasmid forms of pZipneoSV(X) (Z) and pDOL-hGH (D), respectively, were digested with 10 μg of DNA from NIH 3T3 cells (3T3). In the remaining lanes, (Z) and (D) refer to the virus produced by the cells (ψAM) and the virus used to infect the strains of keratinocytes (AY and YF19). (**C**) Northern blot of total RNA (5 μg) was probed with ^{32}P-labeled *neo* gene sequences. Lanes are labeled to indicate the cells from which the RNA was isolated. Abbreviations: ss, splice site; A_n, polyadenylation site; C, control uninfected cells.

To determine whether the ZipneoSV(X) and DOL-hGH proviruses were transcriptionally active in transduced keratinocytes, we analyzed total RNA prepared from G418-resistant cells by Northern blotting (Fig. 1C). Proviral transcripts were detected by hybridization with ^{32}P-labeled *neo* sequences. The expected transcripts from the ZipneoSV(X) provirus were detected in RNA from ψAM2275 cells, infected 3T3 cells, and from the two strains of infected keratinocytes tested (*4*). Two major *neo*-containing RNAs are also expressed in DOL-hGH–infected cells. The larger band represents the full-length transcript that encodes hGH. The smaller band represents the transcript that is initiated by the SV40 early promoter and encodes the *neo* gene product. From the intensities of the bands, it is clear that the ZipneoSV(X) and DOL-hGH proviruses are transcriptionally as active in the two strains of human keratinocytes as in the NIH 3T3 cells.

The synthesis and secretion of biologically active hormone were shown in several ways. First, the secretion of hGH into the medium was detected by immunoprecipitation (Fig. 2A). Keratinocytes were incubated for 3 hours in methionine-deficient medium containing [^{35}S]methionine; the medium was then collected, floating cells were removed by centrifugation, and antiserum to hGH was added. The hormone-antibody complex was precipitated with the aid of *Staphylococcus aureus* (*7*). The ^{35}S-labeled proteins were visualized by fluorography after SDS gel electrophoresis. Human growth hormone with the expected molecular weight of about 22,000 was found in the medium of cultures of both strains of keratinocytes transduced with the hGH gene (Fig. 2A). The secreted hGH was quantitated by specific radioimmune assay (RIA) of portions of medium removed from the culture. The hGH protein was continuously secreted from the transduced keratinocytes for up to 4 days (Fig. 2B). The calculated daily hGH secretion was 11.6 ng per milligram (wet weight); for comparison, the human pituitary gland secretes daily 1.0 μg per milligram of hGH (wet weight) (*8*).

We also measured the biological activity

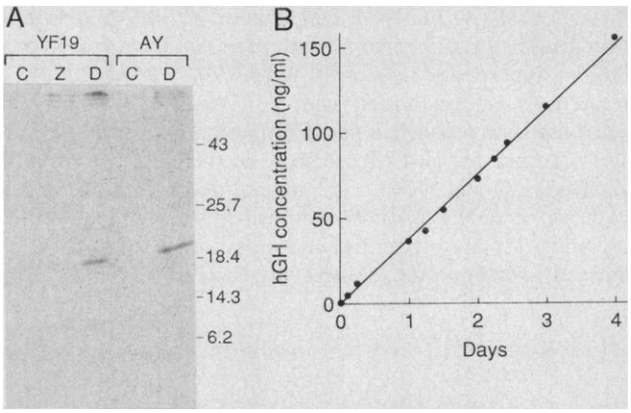

Fig. 2. Secretion of hGH by transduced keratinocytes. (**A**) Immunoprecipitates of [^{35}S]methionine-labeled proteins secreted into the medium by keratinocytes were subjected to electrophoresis on a 15% polyacrylamide SDS gel, and the gel was analyzed by fluorography (*15*). The fluorograph is labeled to indicate the cells from which the labeled medium was harvested and the location of ^{14}C-labeled marker proteins. (**B**) A time course of hGH secretion by keratinocytes transduced with DOL-hGH. Portions (200 μl) of the medium (30 ml) from a confluent culture in a 56-cm^2 dish were removed as indicated over a 4-day period, and hGH was measured by RIA (Hybritech). Abbreviations as in Fig. 1.

of the secreted hGH by its ability to specifically induce differentiation of preadipose 3T3 cells to adipose cells. The extent of adipose differentiation was measured in duplicate cultures of 3T3-F442A cells by an assay for glycerophosphate dehydrogenase (GPD) because this enzyme increases greatly in activity during adipose differentiation (*9*). Confluent monolayers of the 3T3-F442A cells were incubated with purified hGH (2.0 ng/ml) or medium conditioned by DOL-hGH–transduced keratinocytes or by normal keratinocytes. After 12 days, cell extracts were assayed for GPD activity. The hGH synthesized by keratinocytes (2.5 ng/ml, as determined by RIA) was as active in inducing GPD activity as recombinant hGH at the same concentration (1447 and 1164 units per milligram of cell protein, respectively). Medium conditioned by normal keratinocytes did not induce GPD above the background level (102 unit/mg).

Keratinocytes that secreted hGH were tested for their ability to form an epidermis when transplanted to an athymic mouse. Normal or transduced keratinocytes were grown to confluence in 35-mm dishes. The epithelial sheet was detached with Dispase (*10*) and grafted to the subcutaneous tissue of the back of the athymic mouse with the basal side facing outward and was thus completely surrounded by the body fluids of the mouse (*11*). After 1 to 3 weeks, the grafts were removed, snap-frozen or fixed in Formalin, and processed for histological examination. The epidermis resulting from keratinocytes transduced with DOL-hGH (strains YF17, YF19, and AY) revealed a stratified epidermis several layers thick with well-defined stratum granulosum and stratum corneum (Fig. 3). These layers are usually absent from cultured epithelium, and their presence in the regenerated epidermis indicates more complete terminal differentiation. The morphology of grafts formed by transduced keratinocytes was indistinguishable from that of grafts formed by normal keratinocytes.

The growth hormone content of the grafted epidermis was then compared with that of the cultures before grafting. The cultured epithelium was washed, and an extract was made and found to contain 2.2 ng of hGH by RIA. This value is 1.4% of the daily output of hGH by the epithelium while in culture. Seven days after the grafting, the regenerated epidermis from a similar culture contained an average of 4.8 ng of hGH (Table 1). Since the epidermis is thicker than the cultured epithelium, the values shown may include trapped extracellular hormone as well as intracellular hormone. The fact that the value is not much higher than the corresponding value for cultured epithelium may indicate that most of the synthesized hormone left the site of the graft. However, when serum obtained from mice 7 days to 3 weeks after they received grafts was tested for hGH by RIA, none could be detected. This assay is specific for hGH with a sensitivity of 0.2 ng/ml.

We have shown in these studies that murine retroviral vectors can be used to transfer exogenous DNA sequences into human keratinocytes. Although the efficiency of gene transfer was low (approximately 0.5% of the keratinocytes that formed colonies), transduced cells could be selectively propagated owing to their resistance to the drug G418, conferred by the expression of the inserted *neo* gene. Except for a reduced lifetime in culture that seems to have resulted from the exposure to G418, the transduced cells were not obviously altered. After transplantation as an epithelial sheet onto athymic mice, they showed normal ability to generate epidermis and to undergo terminal differentiation.

In contrast to previous studies that indicated that retroviral transcriptional signals are inefficiently utilized in a variety of developmental contexts (*12*), the DOL-hGH retrovirus was as transcriptionally active in cultured keratinocytes as in NIH 3T3 cells and resulted in the secretion of biologically active hGH. This indicates that human keratinocytes have a secretory apparatus adequate for processing and exporting secretory proteins characteristic of other cell types.

Unfortunately, it was not possible to determine the rate of diffusion of hGH from the graft site to the bloodstream, because of the low sensitivity of the hGH RIA. Nevertheless, the ability to express foreign DNA sequences in human keratinocytes raises the question whether genetically engineered skin grafts could be used for systemic delivery of proteins of medical interest now available through the use of recombinant DNA. Current methods require the large-scale purification of these proteins from bacterial or mammalian sources and a suitable method of administration. The use of skin grafts to stimultaneously produce and deliver such proteins might provide an alternative.

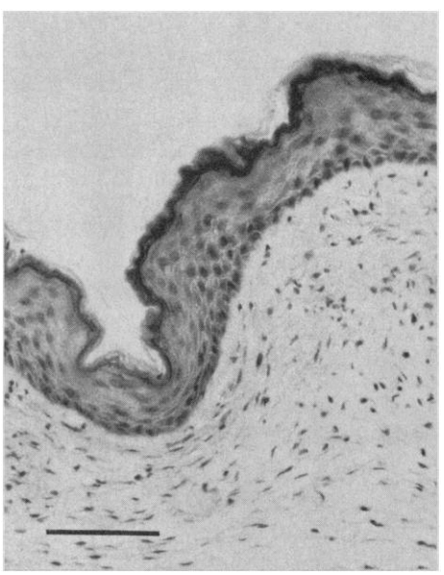

Fig. 3. Formation of an epidermis by grafting transduced keratinocytes. A confluent stratified epithelium formed by keratinocyte strain AY transduced with DOL-hGH was detached intact, by means of Dispase (2.5 mg/ml) and grafted onto an athymic mouse (strain NIH Swiss *Nu/nu*, Taconic Farms) as follows: a surgical flap was made of the dorsal skin of the mouse, and the detached cultured epithelium was applied to the inner surface of the flap with the basal layer facing the vascularized connective tissue (*11*). The flap was then restored to its original position, thus completely enclosing the graft, and the wound was closed with clips. One week after grafting, the epidermis was removed and fixed in Formalin. Paraffin-embedded sections (5 μm thick) were stained with hematoxylin and eosin, which revealed well-developed basal and spinous layers. A granular layer and stratum corneum are also present (scale bar, 100 μm).

Table 1. Detection of hGH in grafted epidermis generated from transduced keratinocytes. Epidermis generated by transduced keratinocytes was harvested 7 to 8 days after grafting, snap-frozen in liquid nitrogen, and powdered. The powder was suspended in 300 μl of 25 m*M* tris buffer (*p*H 7.5), containing 1 m*M* EDTA, and sonically disrupted for 10 seconds at 50 W. The suspension was centrifuged for 5 minutes at 15,000*g*, and the supernatant was assayed for hGH by RIA. YF17 no. 1 and no 2 are control samples of human epidermis generated by uninfected keratinocytes.

Strains	Amount of hGH per graft (ng)	Area of graft (mm^2)
Control		
YF17 no. 1	0	240
YF17 no. 2	0	140
Experimental		
YF19 DOL-hGH	5.6	160
YF17 DOL-hGH	4.0	200

The feasibility of delivering a secreted protein by skin grafting will depend not only on the rate of its synthesis in the graft, but also on the efficiency of its transfer to the circulation. Although, it is known that even quite large proteins can pass in the reverse direction from the dermis into the epidermis (*13*), there is no information on the efficiency of transfer. Further research is being devoted to improved expression of hGH by the use of other retroviral constructs, and to the study of other secreted proteins assayable at lower concentration. In this way we should be able to definitively assess the feasibility of hormone delivery by skin grafting.

REFERENCES AND NOTES

1. J. G. Rheinwald and H. Green, *Cell* **6**, 331 (1975).
2. G. G. Gallico III, N. E. O'Connor, C. C. Compton, O. Kehinde, H. Green, *N. Engl. J. Med.* **311**, 448 (1984).
3. R. D. Cone and R. C. Mulligan, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 6349 (1984).
4. C. L. Cepko, B. E. Roberts, R. C. Mulligan, *Cell* **37**, 1053 (1984); A. J. Korman, J. D. Frantz, J. L. Strominger, R. C. Mulligan, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 2150 (1987).
5. D. A. Williams, I. R. Lemischka, D. G. Nathan, R. C. Mulligan, *Nature (London)* **310**, 476 (1984).
6. R. Mann, R. C. Mulligan, D. Baltimore, *Cell* **33**, 153 (1983).
7. S. W. Kessler, *Methods Enzymol.* **73B**, 442 (1981).
8. U. J. Lewis *et al.*, *Human Growth Hormone*, S. Raiti and R. A. Tolman, Eds. (Plenum, New York, 1986), pp. 439–447; J. B. Martin, *ibid.*, p. 303.
9. M. Morikawa, T. Nixon, H. Green, *Cell* **29**, 783 (1982).
10. H. Green, O. Kehinde, J. Thomas, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5665 (1979).
11. Y. Barrandon, V. Li, H. Green, in preparation.
12. D. Jähner *et al.*, *Nature (London)* **298**, 623 (1982).
13. R. E. Nordquist, R. L. Olson, M. A. Everett, *Arch. Dermatol.* **94**, 482 (1966); E. Schreiner and K. Wolff, *Arch. Klin. Exp. Dermatol.* **235**, 78 (1969).
14. A. P. Feinberg and B. Vogelstein, *Anal. Biochem.* **132**, 6 (1983).
15. The antiserum to hGH NIAMDD [anti-hGH-1 (AFP-4793B)] was obtained from the National Institute of Diabetes and Digestive and Kidney Diseases.
16. We thank H. Goodman for the complementary DNA encoding hGH, and C. Compton for providing histological sections and advice. We thank Genentech, Inc., for the cloned hGH used for controls, and Chiron, Inc., for the cloned human EGF used for cultivation of keratinocytes and 3T3-F442A cells. Supported by NIH grants CA38497 (R.C.M.), CA26712 (R.C.M.), CA40029 (H.G.), and GM33158 (H.G.) and a postdoctoral fellowship from the Cancer Research Institute (J.R.M.)

30 March 1987; accepted 22 June 1987

Uromodulin (Tamm-Horsfall Glycoprotein): A Renal Ligand for Lymphokines

CATHERINE HESSION, JEAN M. DECKER, ANNE P. SHERBLOM, SATISH KUMAR, CHO C. YUE, ROBERT J. MATTALIANO, RICHARD TIZARD, ERIC KAWASHIMA, URSULA SCHMEISSNER, SHERYL HELETKY, E. PINGCHANG CHOW, CYNTHIA A. BURNE, ALAN SHAW, ANDREW V. MUCHMORE*

The protein portion of the immunosuppressive glycoprotein uromodulin is identical to the Tamm-Horsfall urinary glycoprotein and is synthesized in the kidney. Evidence that the glycoproteins are the same is based on amino acid sequence identity, immunologic cross-reactivity, and tissue localization to the thick ascending limb of Henle's loop. Nucleic acid sequencing of clones for uromodulin isolated from a complementary DNA bank from human kidney predicts a protein 639 amino acids in length, including a 24–amino acid leader sequence and a cysteine-rich mature protein with eight potential glycosylation sites. Uromodulin and preparations of Tamm-Horsfall glycoprotein bind to recombinant murine interleukin-1 (rIL-1) and human rIL-1α, rIL-1β, and recombinant tumor necrosis factor (rTNF). Uromodulin isolated from urine of pregnant women by lectin adherence is more immunosuppressive than material isolated by the original salt-precipitation protocol of Tamm and Horsfall. Immunohistologic studies demonstrate that rIL-1 and rTNF bind to the same area of the human kidney that binds to antiserum specific for uromodulin. Thus, uromodulin (Tamm-Horsfall glycoprotein) may function as a unique renal regulatory glycoprotein that specifically binds to and regulates the circulating activity of a number of potent cytokines, including IL-1 and TNF.

UROMODULIN IS AN 85-KD IMMUNOsuppressive glycoprotein that was originally purified to homogeneity from urine of pregnant women on the basis of its inhibitory activity in vitro in an antigen-specific human T-cell proliferative assay (*1*). Subsequent studies demonstrated that uromodulin also inhibits the comitogenic effect of interleukin-1 (IL-1) on both C3H-HEJ thymocytes and the IL-1–responsive D10-G.4 cell line (*2*). By means of a solid-phase binding assay, it was shown that murine IL-1α is a high-affinity ligand for uromodulin with an estimated dissociation constant of $3 \times 10^{-10} M$ (*3*). Although uromodulin is enriched in urine from pregnant women, it has also been isolated from urine of normal male donors; however, uromodulin from the latter source exhibits lower levels of immunosuppressive activity in vitro (*4*). Since the immunosuppressive activity of uromodulin (i) is resistant to pronase digestion, (ii) is destroyed by periodate treatment, (iii) is resistant to a number of protein denaturation procedures, and (iv) can be recovered in the carbohydrate fractions released after digestion of uromodulin with *N*-glycanase, we have suggested that the carbohydrate portion of uromodulin plays a fundamental role in its biologic activity (*5*). Other work showing that oligosaccharides derived from uromodulin or even defined monosaccharides can compete with uromodulin for binding to IL-1 suggests that IL-1 is a "mammalian lectin" with specificity toward carbohydrate moieties on uromodulin (*6*). We now report further characterization of uromodulin, including its amino acid sequence and binding specificity.

Amino acid sequence analysis was performed with homogeneous uromodulin (*1*). In addition to the intact uromodulin polypeptide, tryptic fragments of both *S*-β-(4-pyridylethyl)-uromodulin (*S*-PE-uromodulin) and *N*-succinyl, *S*-PE-uromodulin were

C. Hession, R. J. Mattaliano, R. Tizard, E. Kawashima, U. Schmeissner, S. Heletky, E. P. Chow, C. A. Burne, A. Shaw, Biogen Research Corporation, Cambridge, MA 02142.
J. M. Decker, A. P. Sherblom, A. V. Muchmore, Metabolism Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.
S. Kumar, University of Chicago, Pritzker School of Medicine, Chicago, IL 60637.
C. C. Yue, Case Western Reserve University, Cleveland, OH 44106.

*To whom reprint requests should be addressed.