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Secretion of human parathyroid hormone from rat pituitary cells infected with a recombinant retrovirus encoding preproparathyroid hormone

(recombinant DNA/signal sequence/ψ-2 cells)

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ABSTRACT **In order to study the functions of precursors to secreted proteins, we expressed cloned DNA encoding human preproparathyroid hormone (preproPTH) in rat pituitary cells. We first constructed a recombinant plasmid containing human preproPTH cDNA and retroviral control signals. This recombinant plasmid was transfected into ψ-2 cells, a packaging cell line that produces Moloney murine leukemia viral particles containing no retroviral RNA. The transfected ψ-2 cells generated helper-free recombinant retrovirus encoding preproPTH, and this recombinant retrovirus was used to infect GH4 rat pituitary cells. Clonal lines of the infected GH4 cells contained copies of the recombinant provirus stably integrated via the long terminal repeats, and the expected RNA transcripts of proviral DNA accumulated in the cytoplasm, although no infectious virus was produced. The infected cells synthesized and processed preproPTH appropriately and secreted PTH in response to thyrotropin-releasing hormone, a secretagogue for GH4 cells. Use of recombinant retrovirus permits the introduction of DNA encoding normal and mutant secreted proteins into a number of cell types specialized for secretion. Analysis of the fate of the resultant proteins will help define the specific molecular interactions involved in transmembrane transport and processing of precursor proteins.**

Precursors of secreted proteins contain sequences that direct the proteins along the secretory pathway (1–3). Studies using cell-free extracts have shown that cytoplasmic signal-recognition particles (4) bind to polysomes that are synthesizing these precursors. The signal-recognition particles then bind to receptors on the rough endoplasmic reticulum (5). Little is understood about how the precursors then cross the membrane of the endoplasmic reticulum and traverse the rest of the secretory pathway.

Recombinant DNA technology provides methods for manipulating the sequences of genes encoding secreted proteins and then expressing these genes in cultured cells. This strategy has already been used to show that amino-terminal "signal" sequences and internal "stop transfer" sequences are required for the proper insertion of membrane-bound proteins into those membranes (6–9). To perform analogous experiments to determine how proteins traverse the entire secretory pathway, the genes encoding secreted proteins can be introduced into cells specialized for secretion.

As a prelude to a detailed genetic analysis of the secretory pathway, we have expressed cDNA encoding a precursor of the human calcium-regulating parathyroid hormone (PTH), preproPTH (10), in GH4 cells. In the parathyroid gland, the 25-amino-acid amino-terminal "pre" sequence of preproPTH is cleaved to generate proPTH, and then the 6-amino-acid "pro" sequence is cleaved, yielding PTH. GH4 cells are a well-characterized clonal rat pituitary cell line that secrete prolactin and growth hormone (11); this secretion can be stimulated by secretagogues such as thyrotropin-releasing hormone (TRH) (12, 13).

Exploiting the retroviral life-cycle, we stably introduced cDNA encoding preproPTH into GH4 cells by infecting GH4 cells with a recombinant retrovirus. Clonal lines derived from the recipient cells synthesized and processed preproPTH appropriately and secreted PTH. TRH stimulated the secretion of PTH as well as prolactin from these cells.

MATERIALS AND METHODS

Plasmid Construction. The methods for preparation of plasmid DNA, cleavage with restriction enzymes, purification of DNA fragments, ligation with T4 DNA ligase, and transfection of *Escherichia coli* were performed as described (14, 15).

Cells. The rat pituitary cell line $GH4C_1$ (11), a gift of A. H. Tashjian, and NIH 3T3 cells were maintained in Dulbecco's modified Eagle's medium containing 10% calf serum in 95% air/5% CO_2. The Cl-C cell line, a clonal NIH 3T3 cell line productively infected with Moloney murine leukemia virus, was a gift of C. Tabin.

DNA Transfection and Viruses. DNA transfections were performed with 10 μg of plasmid DNA (16, 17) by the procedure of Graham and Van der Eb (18) as modified by Parker and Stark (19). Virus infections were performed in the presence of 8 μg of Polybrene per ml for 2.5 hr. Aminopterin was omitted from *gpt*-selection medium (20) prepared for use with GH4 cells. When *neo*-selection medium was used, the antibiotic G418 was present at 400 μg/ml (21). Reverse transcriptase activity was assayed for 12 hr after changing the medium (22).

Protein Labeling and Sequence Determination. To radiolabel cells, 10-cm plates of nearly confluent cells were rinsed in methionine-deficient medium containing 5% dialyzed calf serum and then were incubated with 0.5 mCi (1 Ci = 37 GBq) of [S^{35}]methionine (1000 mCi/mM) in 3 ml of medium. Chase medium contained 30 mg of nonradioactive methionine per liter. Incubations were terminated by cell lysis (23). Immunoprecipitation with rabbit anti-bovine PTH antibody or with rabbit anti-rat prolactin antibody was carried out overnight at 4°C. Recovery of the immunoprecipitated proteins was accomplished by the method of Kessler (24). Proteins were separated on a 15–20% acrylamide slab gel containing $NaDodSO_4$; processing of the separated proteins including

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Abbreviations: PTH, parathyroid hormone; cfu, colony-forming units; kb, kilobase(s); TRH, thyrotropin-releasing hormone; LTR, long terminal repeat; MSV, murine sarcoma virus.

sequencing has been described (25). Concentrations of PTH were determined by RIA (26) using chicken antiserum raised against human PTH-(1–34). Prolactin concentrations were also determined by RIA (27).

Preparation and Analysis of DNA and RNA. Cytoplasmic polyadenylylated RNA was isolated from cells as reported (23) except that RNA was not treated with DNase. Virion RNA was prepared by extraction from virus particles banded in sucrose step gradients (28). After denaturation with glyoxal (29), RNA was fractionated by electrophoresis through 1.4% agarose gels, transferred to nitrocellulose, and hybridized with P^{32}-labeled DNA probes (30). Southern blot hybridization was performed as described (14).

RESULTS

Construction of pMSV-hPTH-gpt and Use of ψ-2 Cells to Generate Helper-Free Recombinant Retrovirus. Human PTH cDNA and the selectable marker, *E. coli gpt* were introduced into the previously described (17) retroviral vector pMSV-gpt (Fig. 1). In this construction the PTH sequence replaces the retroviral *gag–pol* sequence, and the *gpt* gene replaces the *env* gene. We expected the retroviral sequences encoding splice donor (5′ SS) and splice acceptor (3′ SS) would allow for the production of a spliced messenger RNA expressing *gpt* (Figs. 1 and 2*A*). To allow the study of secretion of PTH from cells free of budding retrovirus, the *gag*, *pol*, and *env* genes were omitted from the vector pMSV-hPTH-gpt. The packaging cell line, ψ-2, was used to provide the proteins needed for generating infectious virus from RNA transcripts of pMSV-hPTH-gpt.

ψ-2 cells are NIH 3T3 cells that constitutively produce wild-type retroviral proteins by virtue of the fact that these cells have been transfected with DNA encoding a modified Moloney murine leukemia virus. The modification in this viral DNA consists of a deletion of the ψ or packaging sequence, that region required for the encapsidation of viral RNA into virion (17). When a vector such as pMSV-hPTH-gpt, which contains an intact packaging sequence, is transcribed into RNA within the ψ-2 cell, the recombinant RNA can be encapsidated by using the proteins generated from the modified Moloney murine leukemia virus DNA and bud from the cell. Such pseudotyped recombinant retrovirus is competent to infect cells susceptible to infection by wild-type Moloney murine leukemia virus, leading to reverse transcription and incorporation of the recombinant provirus

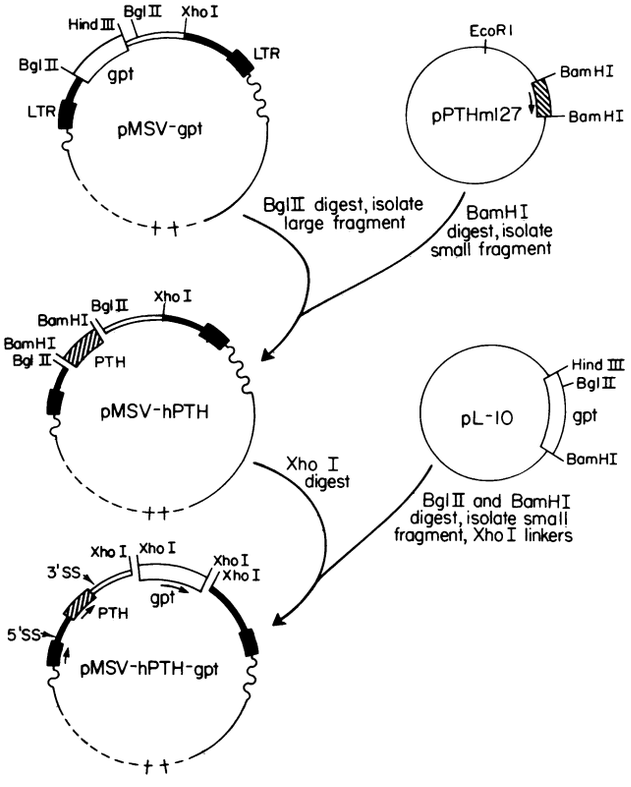

FIG. 1. Construction of pMSV-hPTH-gpt. The representation (not to scale) of the recombinant plasmid pMSV-gpt is as described (17), except that the sequences encoding the splice acceptor site (3′SS), derived from a Moloney murine leukemia provirus, are indicated by double lines (the *Hin*dIII–*Xho* I fragment). Thick lines indicate sequences derived from murine sarcoma virus (MSV) provirus; filled boxes, long terminal repeats (LTRs); wavy lines, mink cellular DNA flanking the recombinant retrovirus; dashed line, polyoma virus early region; thin line, pBR322 sequence; open box, *gpt* gene [the *gpt*-containing plasmid pL-10 has been described (14)]; and hatched boxes, preproPTH insert in pPTHm127 which was derived from pPTHm122 (15) by excising at the *Dde* I and *Hin*fI sites. In pPTHm122, the *Dde* I site is 19 nucleotides upstream of the initiator AUG, and the *Hin*fI site is between the translation termination and polyadenylylation signals. After the ends of the fragment were made flush with DNA polymerase, large fragment, *Bam*HI linkers were ligated to the *Dde* I–*Hin*fI fragment, and the fragment was ligated into the *Bam*HI site of pBR322 to yield pPTHm127. Small arrows indicate transcription orientation.

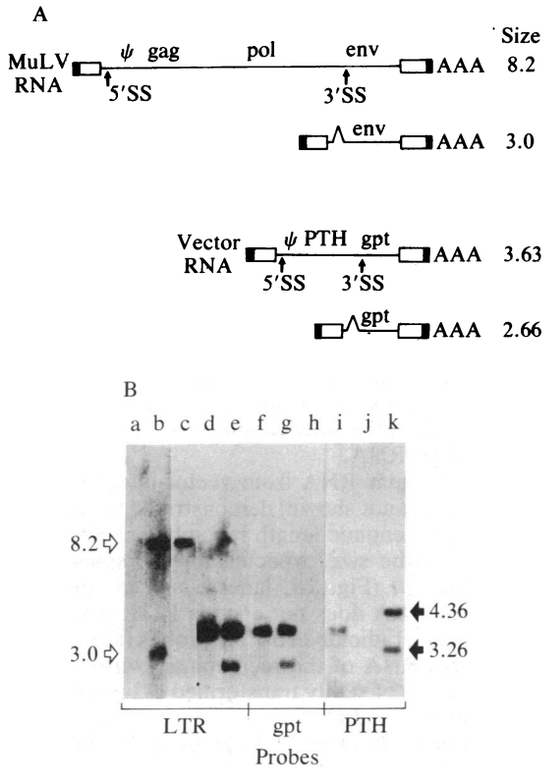

FIG. 2. Analysis of RNA generated in cells infected with recombinant retrovirus. (*A*) Schematic representation of wild-type Moloney murine leukemia virus (MuLV) RNA and expected recombinant retrovirus RNA, including packaging sequence (ψ), 5′SS, 3′SS, and size in kb. (*B*) Blot hybridization of RNA after denaturation with glyoxal and electrophoresis and transfer to nitrocellulose film. Nick-translated ^{32}P-labeled probes were pMSV-hPTH-gpt (lanes a–e) pL-10 (lanes f–h), and pPTHm127 (lanes i–k). The source of RNA in each lane was as follows: a, uninfected NIH 3T3 cells; b, clone Cl-C cells; c, Cl-C cell medium; d, ψ-2 clone 6-2 cell medium; e, 3T3 clone 1 cells; f, ψ-2 clone 6-2 cell medium; g, 3T3 clone 1 cells; h, uninfected NIH 3T3 cells; i, 3T3 clone 1 cells; j, uninfected NIH 3T3 cells; k, DNA size markers. Open arrows indicate Moloney murine leukemia viral RNA sizes (lane b) in kb. Closed arrows indicate denatured DNA size markers (lane k) in kb.

into the cellular genome. However, in the absence of retroviral genes encoding *gag*, *pol*, and *env*, no further infectious retrovirus will be produced by the recipient cells.

Using ψ-2 cells and the plasmid pMSV-hPTH-gpt, we generated helper-free recombinant retrovirus by two procedures: stable transformation and transient rescue (14). In the first procedure, ψ-2 cells were cotransfected with pMSV-hPTH-gpt and pSV2neo (21), and stably transformed clones were isolated by their ability to survive in *neo*-selection medium (21). Cotransfection with pSV2neo was required because ψ-2 cells already contain and express pSV2gpt (17). Of such stably transformed ψ-2 clones surviving *neo* selection, those productive of high titers of recombinant retrovirus encoding *gpt* (and presumably PTH) were determined by assaying for the ability to passage to fresh 3T3 cells the trait of surviving *gpt* selection. Several clones of ψ-2 cells stably transformed with pMSV-hPTH-gpt were productive of 10^6 *gpt* colony-forming units/ml of medium; one such clone, 6-2, was used as a source of recombinant retrovirus.

In the second procedure, transient rescue, ψ-2 cells were transfected with pMSV-hPTH-gpt DNA, and 20 hr later medium from these cells was harvested and used to infect NIH 3T3 cells. Titers of 10^4 colony-forming units/ml were obtained with the vector pMSV-hPTH-gpt.

NIH 3T3 and GH4 cell lines infected with recombinant virus produced by both procedures were shown to be free of infectious retrovirus by RNA blot hybridization analysis (Fig. 2*B*, lanes a and e; also GH4 cell data not shown), by the absence of reverse transcriptase activity in the medium of these cells, and by the inability of medium from such cell lines to passage the *gpt* trait (data not shown). Superinfection of these cells with Moloney murine leukemia virus allowed passage of the *gpt* trait.

Blot Hybridization Analysis of RNA Produced in Cells Infected with Recombinant Virus. Southern blot analysis (data not shown) demonstrated that the retroviral provirus integrated into 3T3 and GH4 cells via the LTRs. From one to several proviruses integrated into each line.

By analogy to wild-type murine leukemia virus (Fig. 2*A*), we predicted that cells infected with the recombinant retrovirus derived from the pMSV-hPTH-gpt vector would generate two RNA species: a smaller spliced RNA of 2.66 kilobases (kb) encoding the selectable marker, *gpt*, and a larger unspliced 3.63-kb RNA.

Analysis of cellular RNA from vector-infected NIH 3T3 (or GH4 cells, data not shown) demonstrated the presence of both the 3.63-kb genomic length recombinant RNA and the smaller RNA of the size expected for the spliced 2.66-kb RNA encoding *gpt* (Fig. 2*B*, lanes e and g). Because the smaller spliced RNA does not encode PTH, it was not detected when using radiolabeled PTH probes (Fig. 2*B*, lane i). Genomic length RNA of the recombinant virus was identified in the medium of stably transformed ψ-2 producer line 6-2 (Fig. 2*B*, lanes d and f).

Processing and Secretion of PTH by Cells Infected with Recombinant Retrovirus. GH4 cells or NIH 3T3 cells, containing recombinant provirus, were pulse-labeled with [S^{35}]-methionine for 15 min and then chased with medium containing excess nonradioactive methionine for the times indicated in Fig. 3. In cells containing recombinant provirus, the predominant product recognized by PTH antiserum at the end of the pulse was proPTH (Fig. 3 *A* and *C*, lane d). No protein comigrating with preproPTH was found. During the chase, the amount of proPTH diminished, and the amount of PTH increased in the cell; as the PTH content of the cell decreased, the amount of PTH in the medium increased. This pattern of processing and secretion is virtually identical to that seen in the parathyroid gland (31), where very little preproPTH is found because of the rapid conversion of preproPRH to proPTH.

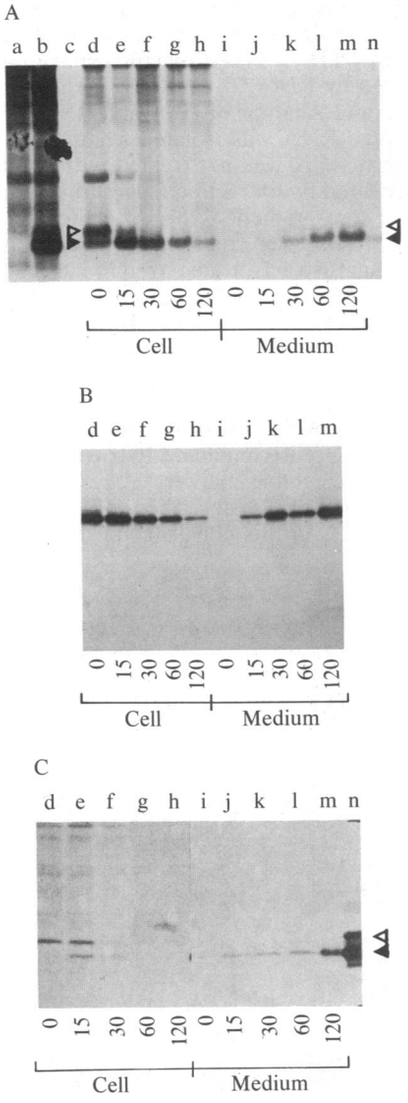

FIG. 3. Analysis of radiolabeled proteins synthesized in cells infected by recombinant retrovirus encoding preproPTH. Pulse–chase experiments were performed as described; proteins were analyzed by autoradiography after overnight immunoprecipitation at 4°C and electrophoresis through polyacrylamide gels containing $NaDodSO_4$. Duration of chase (in min), after a 15-min pulse in the presence of [S^{35}]methionine, is indicated below each lane. At the indicated times, immunoprecipitation was performed on cell extracts or media. (*A*) Immunoprecipitation with PTH antibody. Lanes: d–h, extracts of GH4 cells infected with recombinant retrovirus (GH4 clone 2 cells) after the chase duration indicated; i–m, media from same GH4 clone 2 cells as in lanes d–h after the chase times indicated; a, cell extract of uninfected GH4 cells labeled for 2 hr; b, cell extract of GH4 clone 2 cells labeled for 2 hr. Lanes c and n show radiolabeled proPTH (closed arrow) and PTH (open arrow) markers prepared from human adenoma; these markers became apparent with longer exposure, as in *C*. Five-day autoradiogram exposure. (*B*) Continuation of experiment in *A*. After the immunocomplexes with PTH antibody were removed, cell extracts (lanes d–h) or media (lanes i–m) were subjected to immunoprecipitation with anti-prolactin antibody. Six-hour autoradiogram exposure. (*C*) NIH 3T3 cells infected with recombinant retrovirus (3T3 clone 1 cells); immunoprecipitation of synthesized proteins with PTH antibody. Lanes: d–h, radiolabeled extracts of 3T3 clone 1 cells, chased for the times indicated; i–m, media from the same 3T3 clone 1 cells, chased for the times indicated. Lane n shows radiolabeled proPTH (closed arrow) and PTH (open arrow) prepared from human adenoma. Two-week autoradiogram exposure. Each lane in all figures represents the equivalent of 50% of total cell extract or 25% of medium.

Confirmation of the Identity of proPTH and PTH. To demonstrate the precision of cleavage of preproPTH to proPTH and of proPTH to PTH in GH4 cells, the cleavage products synthesized during a 1-hr labeling of cells with [S^{35}]methionine were eluted from a preparative acrylamide gel and subjected to sequential Edman degradation. Methionine is present at positions 8 and 18 of human PTH and at positions 14 and 24 of human proPTH (10). As can be seen in Fig. 4, the PTH bands from either medium (Fig. 4*A*) or cell extracts (Fig. 4*B*) revealed [S^{35}]methionine at the expected positions 8 and 18; and the material from cell extracts identified as proPTH revealed [S^{35}]methionine at the expected positions 8 and 18; and the material from cell extracts identified as proPTH revealed [S^{35}]methionine at the expected positions 14 and 24 (Fig. 4*C*). Since PTH is the predominant protein synthesized during a 1-hr labeling, it was not surprising that proPTH was contaminated by some PTH (Fig. 4*C*). Similar sequence analysis confirmed the identity of PTH in medium and cell extracts of infected NIH 3T3 cells (data not shown).

Release of PTH and Prolactin from GH4 Cells in Response to TRH. GH4 cells, like normal pituitary cells, release prolactin acutely in response to hormonal stimuli such as TRH (12, 13). We reasoned that, if PTH traversed the same secretory pathway as prolactin, PTH might be released in response to the same stimuli that release prolactin. Table 1 shows the amounts of PTH and prolactin released from GH4 cells containing recombinant provirus after 30 min in response to 100 n*M* TRH. PTH was not detected in the medium of uninfected cells in the presence or absence of TRH. As found by others (12, 13), there was a roughly 2-fold increase in prolactin release in response to TRH. The increase in PTH release is of a similar magnitude, consistent with the notion that PTH and prolactin are sequestered in the same physiologically responsive compartment.

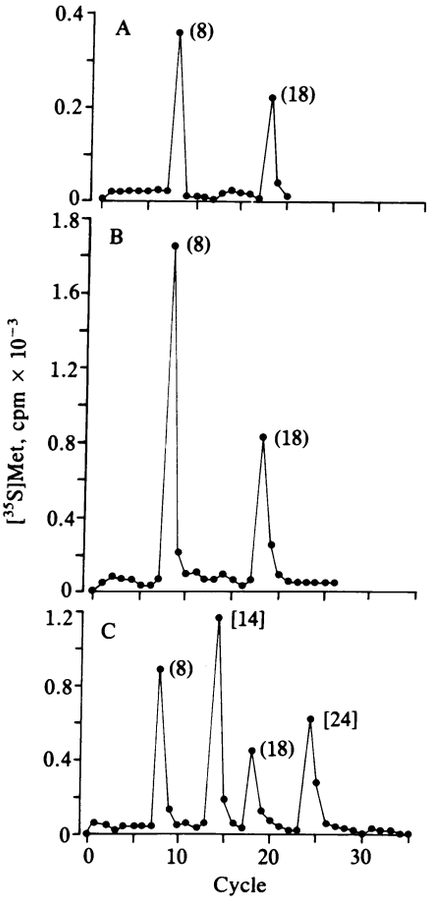

FIG. 4. Sequence analysis of PTH-related proteins. One confluent 10-cm plate of GH4 clone 2 cells was labeled for 1 hr in methionine-deficient medium containing one mCi of [S^{35}]methionine. Immunoprecipitation was performed with PTH antibody on cell extracts or media, and the immunocomplexes were then subjected to $NaDodSO_4$/polyacrylamide gel electrophoresis. Material comigrating with human PTH from medium (*A*) or cell extract (*B*) was eluted and subjected to sequential Edman degradation, as was material comigrating with proPTH from cell extract (*C*). Numbers in parentheses refer to cycle numbers of [S^{35}]methionine peaks consistent with PTH. Numbers in brackets refer to cycle numbers of [S^{35}]methionine peaks consistent with proPTH.

Table 1. Release of PTH and prolactin from infected GH4 cells in response to TRH

TRH addition	Prolactin, ng/ml	PTH, pg/ml
None	206 ± 7	733 ± 47
100 nM	534 ± 61	1549 ± 102
P	<0.01	<0.005

Plates (10-cm diameter) of cells were rinsed twice with culture medium and then incubated for 30 min in 5 ml of medium with or without 100 nM TRH. Plates contained ≈1.8 mg of cell protein. Each hormone concentration in the medium represents the mean from three separate plates ± SEM determined by RIA (26, 27). The *P* values were determined by the two-tailed Student *t* test.

DISCUSSION

We have used the packaging cell line ψ-2 to generate a pure population of recombinant retrovirus encoding human preproPTH and *E. coli gpt*. This recombinant retrovirus was free from helper virus and did not encode wild-type gene products; consequently, fresh cells infected by recombinant retrovirus did not become producers of retrovirus. Results of Southern and RNA blot hybridization analysis were consistent with the integration of proviral DNA into host cells via the retroviral LTRs and subsequent production of the two expected RNA transcripts.

Pulse–chase experiments and amino-terminal sequence analysis of the relevant proteins showed that infected GH4 cells and parathyroid gland slices (10) process and secrete PTH in a similar manner. Moreover, GH4 cells synthesizing preproPTH were induced to release PTH acutely in response to a hormone, TRH, that induces prolactin release from GH4 cells and normal pituitary glands. Presumably, TRH stimulates the secretion of prolactin molecules that have reached specific anatomic compartments, probably the vesicles and secretory granules found in the periphery of GH4 cells (32). The increased secretion of PTH in response to TRH is a physiologic response consistent with the premise that PTH reaches the medium by the same process that prolactin does. This physiologic criterion for secretion will prove particularly useful during the analysis of the secretion of mutant proteins, some of which we have found can be secreted in small, but significant, amounts (unpublished data). Further, the release of PTH from infected GH4 cells in response to TRH supports the hypothesis that secretagogues like TRH stimulate the secretion of any proteins that are located in the same compartment as prolactin rather than stimulate the secretion of prolactin specifically.

Several groups have developed model systems similar to ours that can be used to study how precursors of secreted and membrane proteins are compartmentalized in the cell. DNA encoding growth hormone (33–35) or preproinsulin (36–39) has been introduced into monkey kidney cells or mouse fibroblasts with successful expression, although proinsulin was secreted from these cells without further processing to insulin. In contrast, preproinsulin synthesized in mouse pituitary AtT-20 cells was converted to proinsulin and insulin, and both proinsulin and insulin were secreted

from these cells (39). Insulin alone accumulated in secretory granules of these cells (40). Introduction of modified DNA into monkey kidney cells or mouse fibroblasts in culture has been used to demonstrate the requirement for the amino-terminal "signal" sequences and "stop-transfer" sequences for proper insertion into membranes of viral proteins such as vesicular stomatitis virus protein G (8) and influenza hemagglutinin (6, 7).

The use of defective, helper-free, recombinant retroviruses to infect cells such as rat pituitary GH4 cells offers another model, not previously available, with distinct advantages for studying secretion. Defective recombinant pseudotypes of Moloney murine leukemia virus should be able to infect all cell lines susceptible to infection by Moloney murine leukemia virus. GH4 cells, unlike AtT-20 cells (41), do not bud retrovirus from their plasma membrane, so possible perturbation of the normal secretory pathway by the assembly and budding of virus is avoided. GH4 cells, further, respond to a number of physiologic modulators of prolactin secretion, such as TRH, vasoactive intestinal peptide, and somatostatin (12, 13). Like most cell lines, however, GH4 cells are imperfect replicas of their normal counterparts. GH4 cells contain few secretory granules (32) and, consequently, do not store substantial amounts of hormone intracellularly, for example.

Using this system, we can examine the effect of mutations introduced into the cDNA encoding preproPTH on the movement through the secretory pathway of the resultant mutant proteins. Several deletions of portions of the signal sequence and the adjacent "pro" sequence interfere with secretion; others do not (unpublished data). A detailed analysis of the behavior of these mutants will help define the discrete functions of different portions of the signal sequence and the roles of specific proteins and organelles in the secretory pathway. Further, it should be possible to infect a wide variety of murine cells *in vitro*, as well as rodents and rodent embryos *in vivo*.

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1. Palade, G. (1975) *Science* **189,** 347–358.
2. Milstein, C., Brownlee, G. G., Harrison, T. M. & Mathews, M. B. (1972) *Nature (London) New Biol.* **239,** 117–120.
3. Blobel, G. & Dobberstein, B. (1975) *J. Cell Biol.* **67,** 835–851.
4. Walter, P. & Blobel, G. (1982) *Nature (London)* **229,** 691–698.
5. Meyer, D. I., Krause, E. & Dobberstein, B. (1982) *Nature (London)* **297,** 647–650.
6. Gething, M. J. & Sambrook, J. (1982) *Nature (London)* **300,** 598–603.
7. Sveda, M. M., Markoff, L. J. & Lai, C. J. (1982) *Cell* **30,** 649–656.
8. Rose, J. K. & Bergmann, J. E. (1982) *Cell* **30,** 753–762.
9. Jost, C. S., Hedgpeth, J. & Lingappa, V. R. (1983) *Cell* **34,** 759–766.
10. Potts, J. T., Jr., Kronenberg, H. M. & Rosenblatt, M. (1982) in *Advances in Protein Chemistry*, ed. Anfinsen, C. (Academic, New York), Vol. 35, pp. 323–395.
11. Dannies, P. S. & Tashjian, A. H. (1973) *J. Biol. Chem.* **248,** 6174–6179.
12. Tashjian, A. H., Lomedico, M. E. & Maina, D. (1978) *Biochem. Biophys. Res. Commun.* **81,** 798–806.
13. Westendorf, J. M. & Schonbrunn, A. (1982) *Endocrinology* **110,** 352–357.
14. Mulligan, R. C. & Berg, P. (1980) *Science* **209,** 1422–1427.
15. Hendy, G. N., Kronenberg, H. M., Potts, J. T., Jr., & Rich, A. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 7365–7369.
16. Mulligan, R. C. (1983) in *Experimental Manipulation of Gene Expression*, ed. Inouye, M. (Academic, New York), pp. 155–178.
17. Mann, R., Mulligan, R. C. & Baltimore, D. (1983) *Cell* **33,** 153–159.
18. Graham, R. & Van der Eb, A. (1973) *Virology* **52,** 456–457.
19. Parker, B. A. & Stark, G. R. (1979) *J. Virol.* **31,** 360–369.
20. Mulligan, R. C. & Berg, P. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 2072–2076.
21. Southern, P. J. & Berg, P. (1982) *J. Mol. Appl. Genet.* **1,** 327–341.
22. Goff, S., Traktman, P. & Baltimore, D. (1981) *J. Virol.* **38,** 239–249.
23. Mulligan, R. C., Howard, B. H. & Berg, P. (1979) *Nature (London)* **277,** 108–114.
24. Kessler, S. W. (1975) *J. Immunol.* **115,** 1617–1624.
25. Kronenberg, H. M., Fennick, B. J. & Vasicek, T. J. (1983) *J. Cell Biol.* **96,** 1117–1119.
26. Segre, G. V., Tregear, J. W. & Potts, J. T., Jr. (1976) *Methods Enzymol.* **37,** 38–66.
27. Kieffer, J. D., Mirel, R. D., Mover, H., Erban, J. & Maloof, F. (1978) *Endocrinology* **102,** 1506–1510.
28. Shields, A., Witte, O. N., Rothenberg, R. & Baltimore, D. (1978) *Cell* **14,** 601–609.
29. McMaster, G. K. & Carmichael, G. G. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 4835–4838.
30. Rigby, P. W. J., Dickmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **111,** 237–251.
31. Habener, J. F., Amherdt, M., Ravazzola, M. & Orci, L. (1979) *J. Cell Biol.* **80,** 715–731.
32. Tixier-Vidal, A. (1975) in *The Anterior Pituitary*, eds. Tixier-Vidal, A. & Farquahar, M. G. (Academic, New York), pp. 181–230.
33. Doehmer, J., Barinaga, M., Vale, W., Rosenfeld, M. G., Verma, I. M. & Evans, R. M. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 2268–2272.
34. Pavlakis, G. N., Hizuka, N., Gorden, P., Seeburg, P. & Hamer, D. H. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 7398–7402.
35. Robins, D. M., Pack, I., Seeburg, P. H. & Axel, R. (1982) *Cell* **29,** 623–631.
36. Gruss, P. & Khoury, G. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 133–137.
37. Lomedico, P. T. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 5798–5802.
38. Laub, O. & Rutter, W. J. (1983) *J. Biol. Chem.* **258,** 6043–6050.
39. Moore, H. P. H., Walker, M. D., Lee, F. & Kelly, R. B. (1983) *Cell* **35,** 531–538.
40. Gumbiner, B. & Kelly, R. B. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 318–322.
41. Gumbiner, B. & Kelly, R. B. (1982) *Cell* **28,** 51–59.