cially useful (21). Collaborative programs have clearly buttressed the efforts of developing countries to help themselves.

Although networking is now used in various scientific endeavors, such as medicine, agriculture provided an early seed bed for the concept and most international networks are concerned with crop or livestock production. Breakthroughs in agricultural research have generally come from a combination of cooperation and competition. In food crops, collaboration is widespread, particularly in testing germplasm and devising improved agronomic techniques. In the marketing of finished cultivars, on the other hand, firms often compete for a share of the market; such competition benefits farmers and consumers (22).

Whereas most crop networks currently serve only the major cereals and root crops, other collaborative teams are likely to assemble to upgrade the productivity and yield stability of minor crops, such as most millets and tuber cultigens, that are locally important sources of food and cash in the Third World. Networks are also forming to advance research on microorganisms, such as bacteria involved in nitrogen fixation and yeasts important in fermentation processes.

The networking concept, then, will continue to permeate virtually all aspects of agricultural research, to the enduring benefit of farmers and consumers.

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RESEARCH ARTICLE

Infectious and Selectable Retrovirus Containing an Inducible Rat Growth Hormone Minigene

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Expression of the growth hormone (GH) gene in animals is restricted to the somatotrophic cells of the anterior pituitary, and is transcriptionally regulated by glucocorticoids, thyroid hormone (T_3), and the hypothalamic peptide growth hormone–releasing factor (GRF) (1). To study its hormonal and developmental control we have introduced the cloned GH gene into cultured cells by DNA-mediated gene transfer and into transgenic animals by microinjection into fertilized eggs (2, 3). Because the efficiency of introducing this gene into various cells by transfection is low, and since microinjection is technically difficult, we explored the possibility of creating a retroviral vector that would allow the efficient transfer of a functional GH gene into cultured cells, and possibly fertilized mouse eggs, preimplantation blastulas, and somatic tissue.

The structure and mode of propagation of retroviruses makes them ideally suited for gene transfer (4, 5). These features include efficient transmission to recipient cells, integration into host chromosomal DNA, plasticity of the viral genome for accommodation of foreign DNA, and the ability to infect a wide variety of cell types from many animal species. Selectable genes expressed by retroviral regulatory elements have been successfully propagated (5, 6), but the utility of such vectors would be greatly extended if nonselectable genes expressed from independent promoters could also be transferred. Thus, the many advantages of retroviral gene transfer could be utilized for the study of fundamental aspects of eukaryotic gene expression.

We describe the construction of a selectable retroviral vector containing a rat GH minigene. Recovered high titer retrovirus leads to GH synthesis and secre-

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Abstract. *A growth hormone minigene carrying its natural promoter (237 nucleotides of chromosomal DNA) was stably propagated in a murine retrovirus containing hypoxanthine-guanine phosphoribosyltransferase as a selectable marker. Glucocorticoid and thyroid hormone inducibility was transferred with the growth hormone gene. Recombinant virus with titers of 10^6 per milliliter was recovered. This demonstration that retroviruses can be used to transfer a nonselectable gene under its own regulatory control enlarges the scope of retroviral vectors as potent tools for gene transfer.*

tion in infected cells. Furthermore, the GH promoter in this vector is functional and GH synthesis is inducible by both glucocorticoids and thyroid hormone.

Construction of GH transducing vector. We have previously described the construction of a selectable retrovirus (pLPL) which contains the coding sequences for human hypoxanthine-guanine phosphoribosyltransferase (HPRT, E.C. 2.4.2.8) (5). In this vector, a complementary DNA (cDNA) copy of the human gene coding for HPRT is expressed under the transcriptional control of the viral long terminal repeats (LTR's). Infection of HPRT-negative mammalian cells with HPRT-virus generates HPRT-positive colonies at high frequency. A GH minigene was inserted into this selectable retroviral vector (Fig. 1). The GH minigene consists of a full-length cDNA clone of GH linked to 237 nucleotides of upstream sequences of the GH gene (2, 7). The minigene was inserted in both orientations into the HPRT-virus so that its potential transcription would occur in the same direction as the virus or the opposite one (pLPGHL and pLPHGL, respectively; see Fig. 1).

Transfection and rescue of virus containing HPRT and GH genes. DNA constructs representing each orientation of the minigene were introduced into 208F HPRT$^-$ rat cells by calcium phosphate precipitation, and HPRT$^+$ colonies were selected in HAT medium (8) and pooled for analysis. Table 1 shows that these pooled transfectants secrete significant concentrations of GH. Additionally, these transfected cells synthesize higher concentrations of GH after induction with dexamethasone or with dexamethasone plus T_3 (Table 1). Thus the HPRT-GH-virus constructs are capable of directing the synthesis of GH after transfection into cells, and GH production is inducible.

The HPRT-GH-virus constructs are incapable of synthesizing viral proteins, so helper virus is required to recover these replication-defective viruses. We attempted to recover virus containing the HPRT and GH genes by superinfecting the transfected cells with Moloney murine leukemia virus (MoMLV). Both the HPRT-GH-virus and the MoMLV helper virus should be packaged into virions by proteins synthesized by the helper virus. Table 1 shows that infectious virus transmitting the HPRT$^+$ phenotype was indeed rescued from the transfected, MoMLV-superinfected cells. The ratio of HPRT-GH-virus to helper virus was similar to that previously reported for the parent HPRT-virus (5), indicating that the presence of the GH minigene, in either orientation, did not affect virus rescue.
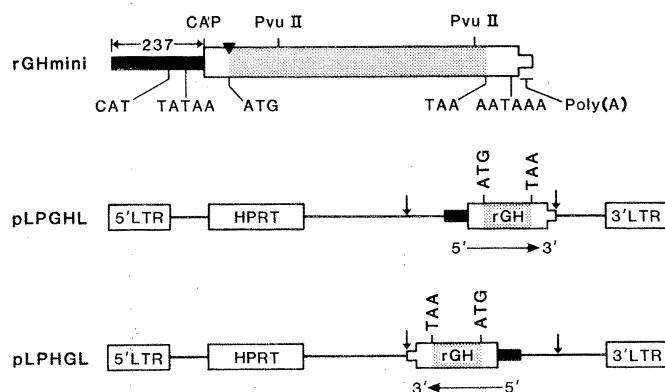

Fig. 1 (left). Structure of the rat GH minigene and selectable retroviral vectors. The rat GH minigene was constructed by fusing the GH promoter region to a full-length cDNA clone as described below. The filled box indicates 5' flanking chromosomal region (237 nucleotides) with putative transcriptional control signals at position −25 (TATAA) and −86 (CAT) relative to the transcription start site (CAP) at position 1. The open box indicates sequences present in GH mRNA with the stipled area representing protein-coding sequences bounded by translation start (ATG) and stop (TAA) sequences. The polyadenylation signal (AATAAA) and 40-nucleotide polyadenylated tail are also shown. The position of restriction endonuclease Pvu II sites used for S_1 nuclease mapping are shown. The 5' Pvu II site is 189 nucleotides from the CAP. The structures of viral vectors containing the GH minigene in the same (pLPGHL) or opposite (pLPHGL) orientation relative to viral transcription are shown. A Bgl II–Pvu II fragment from a GH genomic clone (2) containing 237 nucleotides of 5' flanking sequence and 820 nucleotides of the GH structural gene was subcloned into the Bam HI–Pvu II site of pBR322. Pst I cleaves once in the *amp* gene and once in the first exon of the GH gene of this plasmid. This Pst I fragment was subcloned into a rat GH cDNA clone (pRGH-1) (7) also digested with Pst I. The position of the fusion (the Pst I site) is indicated by a solid triangle. Plasmids carrying the fusion of the chromosomal segment to the cDNA are designated pGHmini. Hind III digestion of pGHmini generates a 1436-nucleotide fragment carrying the entire minigene (1020 nucleotides) and 346 nucleotides of pBR322 DNA. This fragment was blunt-ended with Klenow fragment of DNA polymerase I and inserted into a unique Hpa I site in the retroviral plasmid pLPL (5). The site of insertion is indicated by vertical arrows.   Fig. 2 (right). Flow diagram of experimental approach. HAT^R clones are HAT-resistant.
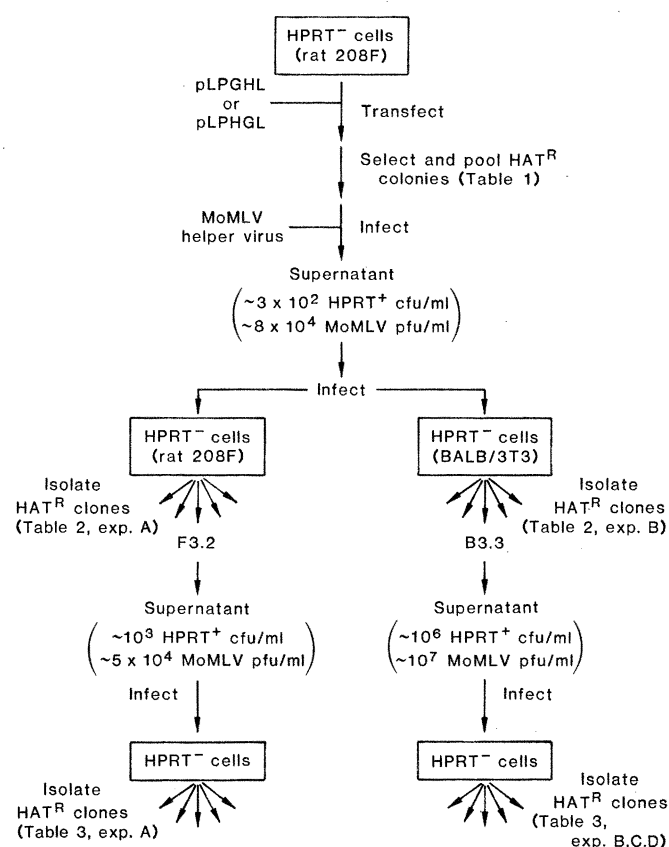
To determine whether the GH minigene was being cotransferred in the recovered virus along with HPRT, we used virus recovered from cells transfected with pLPGHL or pLPHGL to infect 208F rat cells and BALB/3T3 mouse cells (see Fig. 2). Sixteen independent HPRT$^+$ clones were isolated and characterized for GH production. Table 2 shows that of the eight HPRT$^+$ rat clones and the eight HPRT$^+$ mouse clones, at least half in each case synthesized significant amounts of GH. In the remaining infectants, either the minigene was inactive or it failed to cotransfer with the HPRT gene. Two of the rat infectants (clones F0.2 and F3.2, Table 2) were clearly responsive to dexamethasone, and this response was potentiated by T_3. Thus GH expression and regulation appeared to be independent of the transcriptional orientation of the GH minigene. In addition, two of the mouse clones appeared to respond to dexamethasone and T_3 (clones B1.2 and B1.4, Table 2), although the concentrations of GH production were low.

Analysis of viral DNA in infectants. Since retroviruses integrate into cellular DNA in a linear fashion and are flanked by LTR's, the GH minigene should be flanked by viral LTR's even though the integration site in the cellular genome is likely to be random (4). DNA extracted from several viral infectants was cleaved with the restriction enzyme Bam HI, which cuts only once in the HPRT-GH-provirus (between the HPRT and GH DNA sequences), and the resulting fragments were analyzed by the technique of Southern (9). A GH probe hybridized to a single band in uninfected mouse and rat cells, presumably derived from the endogenous GH gene (Fig. 3), while in DNA prepared from five infected clones an additional band was detected. The new fragment had a different size in each of the infectants, as expected, because of the random occurrence of Bam HI sites in the cellular DNA near the virus integration site. Each infectant contained a single integrated provirus, since only one new band was present in DNA from each of the clones. Similar results were obtained when HPRT sequences were used as a probe (data not shown).

Finally, DNA from these clones was digested with Sst I and subjected to the same analysis. Sst I cuts once in each LTR of the virus and should reveal the size of the integrated HPRT-GH-provirus. In DNA from each of the infectants, there was a single new band in addition to the endogenous band (Fig. 3). The new bands were of the same size and match the expected size (5.3 kilobases)

of an integrated HPRT-GH-provirus. Thus, no major rearrangement of the viral genome had occurred during generation of virus or of infection of these clones. We conclude that the simultaneous propagation of HPRT and the GH minigene is independent of the transcriptional orientation of the latter relative to the viral LTR.

Viral RNA analysis. To examine transcription from the HPRT-GH-provirus, we analyzed the RNA by RNA blot and S_1 nuclease techniques. Northern blot analysis of growth hormone messenger

Table 1. Analysis of transfected cells for GH production and virus rescue. Viral DNA (10 μg per 5-cm dish of cells) was introduced into 208F HPRT$^-$ rat cells by calcium phosphate precipitation (14). One day after transfection, the cells were trypsinized and seeded into HAT (30 μM hypoxanthine, 1 μM aminopterin, 20 μM thymidine) medium. HPRT$^+$ colonies were visible in about 5 days and were pooled for analysis. For assay of GH production, the cells were seeded at about 20 percent confluency in growth medium containing 10 percent fetal bovine serum with no added inducers (control) or with $10^{-6}M$ dexamethasone or $10^{-6}M$ dexamethasone and $10^{-7}M$ T_3. After 3 days, GH in the medium was measured with an NIAMDD radioimmunoassay kit and rat GH standard NIAMDD-rGH-I-4 (2). Results are expressed in nanograms of GH per millilter of medium. Induction was calculated as the ratio of GH production with dexamethasone (Dex) + T_3 compared to control (GH production from uninduced cells). Fetal bovine serum contains low levels of inducers of GH production; thus the induction may be underestimated. In a separate experiment, the transfected cells were infected with MoMLV helper virus [MLV-K (15)] and passaged twice, and then virus production was measured in medium harvested after 16 hours from a confluent dish of cells. Colony-forming units (cfu) per milliliter of medium were measured on 208F rat cells (5), and plaque-forming units (pfu) on NIH/3T3 TK$^-$ cells (15, 16).

Transfected DNA	Growth hormone production (ng/ml)			Induction	Virus recovery	
	Control	Dex	Dex + T_3		HPRT$^+$ ($10^2 \times$ cfu/ml)	MoMLV ($10^4 \times$ pfu/ml)
pLPGHL						
Experiment 1	11	23	24	2.2	6.5	8
Experiment 2	22	102	99	4.5	3.2	8
pLPHGL						
Experiment 1	29	83	101	3.5	2.4	7
Experiment 2	35	70	75	2.1	2.3	8
No DNA	<2	<2	<2			

Table 2. Growth hormone production and induction in cells infected with virus recovered from transfected cells. Hormone production from induced or uninduced cells was measured as described in Table 1. HPRT$^+$ colonies induced by virus recovered from transfected 208F cells (Table 1) were isolated by using cloning cylinders. The first letter of the clone designation refers to the cell type of the infectant, F for 208F rat cells or B for BALB/3T3 (17) mouse cells. Induction values that may not be meaningful because of limitations of the assay at low GH levels are indicated in parentheses. Dex, dexamethasone.

Recovered virus	HPRT$^+$ clone number	Growth hormone production (ng/ml)			Induction
		Control	Dex	Dex + T_3	
Experiment A. 208F rat cell infectants					
LPGHL	F0.2	6.0	22	30	5.0
	F0.3	6.5	7.9	8.9	1.4
	F1.2	13	20	20	1.5
	F1.3	3.1	2.4	2.7	(0.9)
LPHGL	F2.1	3.3	3.6	2.9	(0.9)
	F2.4	4.0	3.7	2.4	(0.6)
	F3.2	25	93	126	5.0
	F3.3	4.5	4.4	4.0	(0.9)
Uninfected cells		<1.5	<1.5	<1.5	
Experiment B. BALB/3T3 mouse cell infectants					
LPGHL	B0.2	12	10	12	1.0
	B0.4	12	10	16	1.3
	B1.2	3.5	7.6	8.6	(2.5)
	B1.4	2.7	5.9	6.8	(2.5)
LPHGL	B2.3	<1	<1	<1	
	B2.4	<1	<1	<1	
	B3.3	236	246	289	1.2
	B3.4	<1	<1	<1	
Uninfected cells		<1	<1	<1	

RNA (mRNA) synthesized by the integrated virus in several infected cell lines is shown in Fig. 4A. The major mRNA species produced in all of the infectants producing rat GH was the same size (1 kb) regardless of the transcriptional orientation of the minigene. Furthermore, this transcript was identical to the mRNA product of the endogenous GH gene in rat pituitary cells (Fig. 4A). S_1 nuclease analysis was used to characterize the 5' terminus to determine whether the appropriate GH transcriptional signals were being utilized. The results in Fig. 4B show that mRNA produced from the endogenous GH gene protects a 189-nucleotide fragment spanning the promoter region and identifies a 5' cap site 25 nucleotides from the sequence TA-TAA. A fragment of identical size is protected by mRNA obtained from HPRT-GH-virus–infected mouse or rat cells (Fig. 4B, lanes 3 and 4), thus confirming accurate initiation from the minigene. Furthermore, S_1 nuclease and mRNA size analysis imply that the GH polyadenylation signals are being used.

The GH minigene is inducible. RNA analysis confirmed that the GH minigene, in addition to containing functional initiation and polyadenylation sites, also remained inducible with dexamethasone and T_3. Induction of mRNA (Fig. 4A) paralleled increases in GH production (Table 2). For example, rat clone F3.2 showed a fivefold induction of both GH mRNA and secreted protein after induction. Clone F0.2 showed similar changes in both RNA and GH synthesis. In contrast, mouse clone B3.3 synthesized constitutively and relatively high concentrations of GH, which remained unaffected by hormone administration. Correspondingly high GH mRNA levels also remained unaltered. Some clones, such as B1.4 and F1.2, revealed low levels of both GH RNA and secreted protein. In addition to the GH message synthesized from the minigene, the GH probe should have hybridized to a message corresponding to the viral genome. This transcript is not evident in Fig. 4A. Nonetheless, since these cell lines released infectious HPRT-GH-virus after superinfec-

tion, full-length transcripts must have been present.

Phenotypic variability in primary infectants. We have shown by a variety of criteria that an HPRT-GH-retrovirus can be generated and directs the synthesis of RNA's from two independent transcriptional units. Regulation of GH gene expression, in response to dexamethasone and T_3, was maintained in several of the rat cell infectants and possibly some of the mouse infectants. However, the inducibility and transcriptional activity of the GH minigene was highly variable. To examine the nature of this variability, we further analyzed the virus released from several of the primary infectants to determine if the phenotype was maintained in successive rounds of infection.

Analysis of virus from an inducible infectant. The primary infectant F3.2 synthesized GH at high levels, GH synthesis was inducible, and this clonal cell line contained a single HPRT-GH-provirus of the correct size (Fig. 3). HPRT-GH-virus was rescued from F3.2 cells by
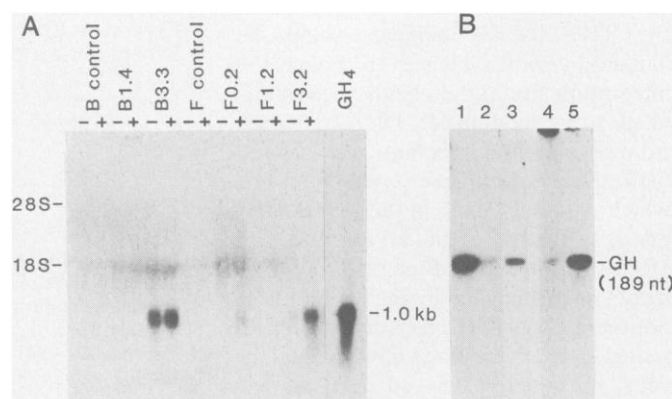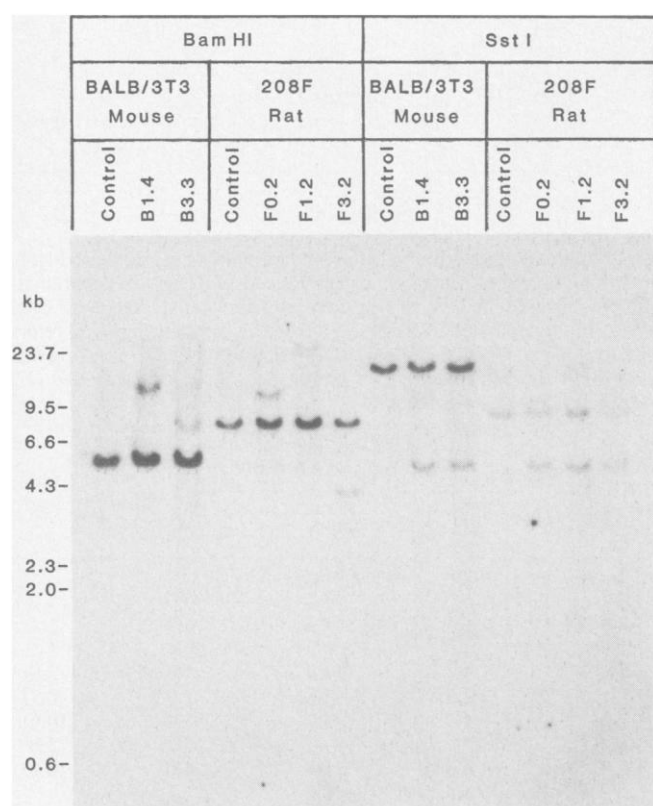

Fig. 3 (left). Analysis of DNA's from uninfected and infected cells with a GH probe. The infected cells are designated as in Table 2. Size markers (Hind III fragments of phage λ DNA) are indicated in kilobases at the left. The DNA's (10 μg per lane) were digested overnight with either Bam HI or Sst I, subjected to electrophoresis, and transferred to nitrocellulose. After prehybridization had occurred, the filter was incubated for 16 hours at 42°C with ^{32}P-labeled GH cDNA (10^6 count/min per milliliter) in the presence of 10 percent dextran sulfate. The filter was washed in 2× SSC (standard saline citrate) and 0.1 percent SDS for 30 minutes at room temperature and exposed to x-ray film.   Fig. 4 (right). Analysis of GH RNA's from infected cells. (A) RNA from uninfected control cells or infected cells grown for 3 days in the absence (−) or presence (+) of $10^{-6}M$ dexamethasone and $10^{-7}M$ T_3 was subjected to electrophoresis, transferred to nitrocellulose, and hybridized with a ^{32}P-GH cDNA probe. Designations of infected cells are as in Table 2. All lanes have 10 μg of total cytoplasmic RNA except for GH$_4$, which has 1 μg of poly(A^+) cytoplasmic RNA. (B) S_1 nuclease analysis of RNA from clones B3.3 and F3.2. Lanes 1, 2, and 5, 100 ng, 10 ng, and 100 ng of poly(A^+) cytoplasmic RNA from GH$_4$ cells; lane 3, 10 μg of total cytoplasmic RNA from clone F3.2; lane 4, 10 μg of cytoplasmic RNA from clone B3.3; lanes 1 and 5, overnight exposure; lane 4, exposure for 3 days; lane 3, exposure for 6 days. F3.2 and B3.3 cells were grown for 3 days in the presence of dexamethasone and T_3 prior to RNA harvest. RNA samples (made up to 10 μg with yeast RNA) were hybridized overnight with 50,000 count/min of kinased 2.8-kb Pvu II fragment (see Fig. 1) of the GH minigene containing 189 nucleotides of coding region, 237 nucleotides of 5' flanking chromosomal DNA, and 2.4 kb of pBR322 DNA. Hybridization was conducted at 56°C in a 30-μl reaction mixture containing 40 mM Pipes (pH 6.4), 1 mM EDTA, 400 mM NaCl, and 80 percent formamide. This was followed by digestion with 250 μg of S_1 nuclease in 280 mM NaCl, 50 mM sodium acetate (pH 4.6), and 4.5 mM ZnSO$_4$ at 37°C for 1 hour. The products were precipitated and analyzed by autoradiography after electrophoresis on 8 percent denaturing polyacrylamide gels. The size of the protected fragment was measured at 189 bases (see Fig. 1) by comparison to ^{32}P-labeled Hinf-digested pBR322.

superinfection with helper virus and used to infect 208F rat cells, as outlined in Fig. 2. Virus released from F3.2 cells had a titer of 10^3 HPRT$^+$ colony-forming units per milliliter, which is similar to that obtained with HPRT-virus in 208F rat cells (5). Analysis of six HPRT$^+$ secondary infectants (Table 3, experiment A) revealed that all HPRT$^+$ clones generated by the virus synthesized GH and responded to both dexamethasone and T$_3$. These results demonstrate concomitant transfer of the HPRT and GH genes by the virus, and show that virus from this primary infectant is relatively homogeneous.

Analysis of virus from a constitutive high expression infectant. The primary infectant B3.3 synthesized GH at constitutively high levels and was unresponsive to dexamethasone or T$_3$ induction. These cells also contained a single HPRT-GH-provirus which, by Southern blot analysis (Fig. 3), did not appear to be rearranged. Both the viral and GH transcription units were functional, since the cells were HPRT-positive and produced high levels of GH mRNA. It was therefore of interest to examine virus rescued from this cell line to determine if constitutive high GH expression was transmissible, or alternatively, due to the cellular or chromosomal environment of the provirus. When HPRT-GH-virus from MoMLV-superinfected B3.3 cells was rescued and used to infect 208F rat cells, all of the HPRT$^+$ colonies examined synthesized GH, and, furthermore, all were inducible by dexamethasone (Table 3, experiment B). In fact, virus from B3.3 cells generated approximately the same phenotype in recipient cells as virus from F3.2 cells (Table 3, experiment A). This result suggests that the virus in B3.3 cells is identical to that in F3.2 rat cells, and that the difference in expression and regulation is mediated by cellular factors.

We also used the same virus rescued from B3.3 cells to infect BALB/3T3 mouse cells. In contrast to the rat infectants, HPRT$^+$ BALB/3T3 secondary infectants had widely variable phenotypes (Table 3, experiment C). Some displayed constitutively low secretion of GH (for example, B3.3B3), others exhibited constitutively high secretion (for example, B3.3B1 and B3.3B4), while others were inducible (for example, B3.3B2). We conclude that relative levels of expression and regulation of the HPRT-GH-virus in BALB/3T3 cells can be strongly influenced by cellular or chromosomal environment.

Analysis of virus from a variant with constitutively low expression. We exam-

ined virus recovered from cells expressing low levels of GH to determine if this property transferred to secondary infectants. HPRT-GH-virus from MoMLV-superinfected clone B3.3B3 cells (Table 3, experiment C) was used to infect 208F rat cells, and HPRT$^+$ colonies were selected. The colonies were pooled to obtain GH synthesis data averaged over many clones. Two independent experiments showed that the virus released from B3.3B3 directed the synthesis of significantly higher levels of GH and GH expression and was now inducible (Table 3, experiment D). Thus, the provirus in B3.3B3 cells was not genetically restricted to constitutively low levels of expression, and in the BALB/3T3 mouse cells provirus expression and regulation were highly variable.

Generation of HPRT-GH-virus. The results presented here demonstrate that a GH minigene carrying its natural promoter can be propagated in a murine retrovirus containing a selectable gene. Virus generated from cells transfected with HPRT-GH-virus constructs was relatively inhomogeneous, in that cells converted to HPRT$^+$ by such virus did not

always secrete GH (Table 2). However, when virus rescued from either rat or mouse primary infectants was used to infect rat cells, all 12 of the resultant HPRT$^+$ clones examined synthesized GH at similar levels and responded to induction (Table 3, experiments A and B), showing that virus rescued from primary infectants containing integrated HPRT-GH-proviruses was homogeneous. Transmissible HPRT-GH-virus with a titer of 10^6 colony-forming units per milliliter could be obtained (Fig. 2). Thus high titer virus obtained from primary infectants retains both genes and these genes do not segregate in subsequent infections. This finding is in contrast to data previously reported where two genetic markers segregated upon propagation of the virus (10).

Expression and regulation of the GH minigene. The utilization of the GH promoter in the minigene is demonstrated by its activity in both transcriptional orientations relative to viral transcription, and by S$_1$ nuclease analysis which reveals that transcripts from either orientation have the same cap site as the endogenous GH gene product. No spe-

Table 3. Growth hormone production and induction in secondary infectants. Cellular clones F3.2, B3.3, and B3.3B3 were superinfected with MoMLV and the resultant virus was used to infect uninfected HPRT$^-$ cells. HPRT$^+$ colonies were isolated by using cloning cylinders and were assayed for GH production and induction as described in Table 1. The first part of the designation for each clone indicates the clone from which virus was isolated (F3.2 or B3.3), the next letter indicates the cell type of the recipient (208F or BALB/3T3), and the last number indicates the serial clone number. In some experiments, HPRT$^+$ colonies were pooled for analysis (that is, without subcloning) to yield average values for growth hormone production. Dex, dexamethasone.

Clone number	Growth hormone production (ng/ml)			Induction
	Control	Dex	Dex + T$_3$	
Experiment A. Virus from clone F3.2. 208F recipient cells				
F3.2F1	75	261	327	4.4
F3.2F2	46	216	258	5.6
F3.2F4	54	131	236	4.4
F3.2F5	58	188	190	3.3
F3.2F6	53	119	152	2.9
F3.2F7	30	47	53	1.8
Pooled infectants	55	143	172	3.1
Experiment B. Virus from clone B3.3. 208F recipient cells				
B3.3F1	41	122	172	4.2
B3.3F2	85	216	191	2.2
B3.3F3	72	151	212	2.9
B3.3F4	39	99	120	3.1
B3.3F5	64	101	191	3.0
B3.3F6	58	113	152	2.6
Pooled infectants	35	79	93	2.7
Experiment C. Virus from clone B3.3. BALB/3T3 recipient cells				
B3.3B1	310	158	230	0.7
B3.3B2	26	81	62	2.4
B3.3B3	12	28	13	1.1
B3.3B4	540	245	260	0.5
B3.3B5	32	41	61	1.9
B3.3B6	100	126	130	1.3
Experiment D. Virus from clone B3.3B3. 208F recipient cells				
Pooled infectants	22		74	3.4
Pooled infectants	33		93	2.8

cific measures were taken to create a functional polyadenylation signal at the 3' end of the GH minigene. The 3' end of the GH minigene contains a 40-nucleotide polyadenylated stretch preceded by 19 nucleotides with the sequence AA-TAAA. The results suggest that these sequences present in cDNA are sufficient to generate a stable polyadenylated 3' terminus. However, they do not demonstrate whether the 3' end is generated by a cleavage event followed by poly-adenylation, or whether the polyadenylated tail itself is transcribed from the insert.

We showed previously (2) that the endogenous GH gene is transcriptionally regulated by both glucocorticoids and T_3. This regulatory capacity is retained by the transduced GH minigene. Both of these inducers act through receptors that are DNA-binding proteins and thus presumably recognize specific DNA sequences. These results suggest that these putative regulatory sequences are present in the GH minigene. We have also shown (2) that the entire rat GH gene can be expressed and regulated in mouse embryo fibroblasts when it is linked to retroviral DNA. Although it has not been formally shown, we believe that transcriptional enhancer elements in the viral LTR's facilitate the expression of the GH gene, since the rat GH gene by itself, upon transfection, is not actively transcribed (data not shown).

Modulation of expression. Serial transduction and rescue of the GH gene indicates that it can be stably propagated but not always predictably expressed. When virus from either rat or mouse primary infectants was used to infect rat cells, all 12 of the HPRT$^+$ examined synthesized similar levels of GH and were inducible. In contrast, when the same virus was used to infect BALB/3T3 mouse cells, GH production

and inducibility in the resultant HPRT$^+$ clones were highly variable. This variation was not due to segregation of the two genes because even cells that produced low levels of GH (for example, B3.3B3) produced virus that directed high GH synthesis in recipient rat cells (Table 3, experiment D). Variation in the expression of the GH gene could be attributed to chromosome position effects, which have been reported to modulate the expression of various retroviral genomes (11). Alternatively, it could be due to other *trans*-acting factors, such as variations in the amount of glucocorticoid or T_3 receptors, that lead to variability in both basal and induced levels of transcription. The availability of cloned cell lines containing single copies of HPRT-GH-provirus should be useful for studying factors influencing the expression of GH and HPRT genes.

We conclude that functional and regulated eukaryotic genes can be efficiently and stably propagated in selectable retroviral vectors. While the selectable marker contained in the retrovirus is necessary for the generation of virus-producing cell lines, the amount of virus obtained from these lines is sufficiently high to permit infection of all cells in a culture dish and eliminate the need for biochemical selection. Retroviruses can also be used as efficient means to transduce DNA into somatic tissue or into the germ line of animals (11). Germ line transmission should be particularly valuable for studying tissue-specific gene expression and investigating the temporal regulation of gene activity during development (11). To date, gene function following germ line transformation has been studied extensively only in *Drosophila* and mice (12). We have been able to produce high titer HPRT-GH-virus in the absence of helper virus using a broad host range amphotropic packag-

ing system (13). By means of such a system it should be possible to introduce retroviruses into embryonic and somatic tissue of a wide variety of animal species.

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17. HPRT$^-$ BALB/3T3 cells (B77/OTG), which were transformed with Rous sarcoma virus and then selected for resistance to ouabain and 6-thioguanine, were a gift from H. Ozer.
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