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# Human Growth Hormone as a Reporter Gene in Regulation Studies Employing Transient Gene Expression

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**The human growth hormone (hGH) transient assay system described here is based on the expression of hGH directed by cells transfected with hGH fusion genes. Levels of secreted hGH in the medium, measured by a simple radioimmunoassay, are proportional to both levels of cytoplasmic hGH mRNA and the amount of transfected DNA. The system is extremely sensitive, easy to perform, and is qualitatively different from other transient expression systems in that the medium is assayed and the cells themselves are not destroyed. The hGH transient assay system is appropriate for analyses of regulation of gene expression and was utilized here to investigate the effect of the simian virus 40 enhancer on the herpes simplex virus thymidine kinase promoter and the effect of zinc on the mouse metallothionein-I promoter. The expression of hGH can also be used as an internal control to monitor transfection efficiency along with any other transient expression system. All cell types tested thus far (including AtT-20, CV-1, GC, $GH_4$, JEG, L, and primary pituitary cells) were able to secrete hGH into the medium.**

The analysis of expression of DNA sequences introduced into cells in culture has provided important insights into the mechanisms which govern eucaryotic gene expression. Initially, these studies relied on the creation of new clonal cell lines which contained exogenously added DNA sequences stably integrated into their genomes at random chromosomal positions (21). Because the generation of such cell lines is a long and laborious process and the varying chromosomal contexts of the integrated DNA have different effects on its expression (7), this approach has largely been supplanted by strategies which employ the analysis of gene expression relatively soon after introduction of the new DNA sequences into large pools of cells.

Measurement of gene expression in such transient systems is based on determining the levels of either RNA or protein whose synthesis was directed by the transfected DNA. The use of vectors directing the synthesis of relatively easily assayable enzymatic activities, particularly the bacterial enzyme chloramphenicol acetyltransferase (CAT) (8), has greatly facilitated the study of gene expression in transiently transfected cells (11). The advantages of the CAT assay are its reliability, rapidity, quantifiability, and low intrinsic background. We describe here a series of new vectors which are based on a simple immunological detection of human growth hormone (hGH) secreted by the transfected cells. We show that these vectors respond appropriately to previously characterized regulatory effectors. A direct comparison of the hGH transient expression system with the CAT system in mouse L cells demonstrates that the hGH system is at least 10 times more sensitive and possesses all the other advantages of the CAT system. Furthermore, the hGH system is qualitatively different from generally utilized systems in that it does not require destruction of the transfected cells, thus allowing continuous monitoring of transient expression from a cell population.

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## MATERIALS AND METHODS

**Plasmid constructions.** A mouse metallothionein-I (mMT-I)-hGH fusion gene was constructed by ligating a 1.8-kilobase *Eco*RI-*Bgl*II fragment containing promoter sequences from the mMT-I gene (9) to a 2.1-kilobase *Bam*HI-*Eco*RI hGH gene fragment which contains structural sequences from the start of transcription at the *Bam*HI site to 526 base pairs past the poly(A) addition site (5). The ligated material was digested with *Eco*RI and inserted into the *Eco*RI site of the plasmid vector pUC12 (20). The resulting plasmid is referred to as pXGH5.

A general vector for the study of promoter regulation employing the secretion of hGH as an assay was constructed by ligating the 2.1-kilobase *Bam*HI-*Eco*RI fragment containing the hGH structural sequences into the *Bam*HI-*Eco*RI sites of pUC12. This 4.8-kilobase plasmid, lacking any known eucaryotic promoter, is referred to as p0GH (zero, i.e., no promoter) and is suitable for the insertion of eucaryotic promoter sequences of interest. The presence of the pUC12 polylinker immediately upstream of the hGH sequences should facilitate such constructions.

A thymidine kinase (TK)-hGH fusion gene was constructed by ligating a 200-base-pair *Pvu*II-*Bgl*II fragment containing promoter sequences from the herpesvirus TK gene (14) into the *Hin*cII-*Bam*HI sites of the p0GH polylinker. The resulting plasmid is referred to as pTKGH. An approximately 200-base-pair fragment containing the two 72-base-pair repeats of simian virus 40 (SV40) was removed from pSV2Agpt-LR (kindly provided by Ulla Hansen) by *Bam*HI digestion, converted to a *Hin*dIII fragment by the addition of *Hin*dIII linkers, and inserted into the *Hin*dIII site of pTKGH. The resulting plasmid is referred to as pSVTKGH and is suitable for the study of the effects of SV40 enhancer sequences on pTKGH expression.

Both p0GH and pTKGH were constructed from fragments of DNA whose complete sequence is available from a variety

of data bases. A portion of pXGH5 upstream of the mMT-I promoter is not yet sequenced. Standard techniques of molecular cloning utilized to construct and isolate these plasmids were performed as described previously (12).

**Tissue culture.** L cells were grown in Dulbecco modified eagle medium supplemented with 10% calf serum, and XC cells were grown in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum. After transfection, the medium was changed every 3 to 4 days.

**Transfections.** Approximately $5 \times 10^5$ L cells or XC cells were plated on 10-cm tissue culture dishes and allowed to grow for 3 days to approximately 30% confluence. DEAE-dextran-mediated transfections were performed essentially as described previously (11) with the use of Nuserum (Collaborative Research, Inc., Waltham, Mass.) during the 4-h transfection (S. Aruffo and B. Seed, personal communication). Briefly, the medium was aspirated from the dish, and the cells were washed once with phosphate-buffered saline. The desired amount of DNA was ethanol precipitated and suspended in 60 μl of Tris-buffered saline (0.15 M NaCl, 5 mM KCl, 1.5 mM $Na_2HPO_4$, 2.5 mM Tris-base, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, adjusted to pH 7.5 [the $CaCl_2$ and $MgCl_2$ were prepared together as a 100× stock and added slowly to the other components to avoid precipitation]) and then added to 120 μl of warm (30 to 40°C) 5-mg/ml DEAE-dextran in Tris-buffered saline. This mixture was then combined with 3 ml of 10% Nuserum and added to the plate. After incubation for 4 h at 37°C in 5% $CO_2$ the DNA–DEAE-dextran–Nuserum was removed and replaced by 5 ml of 10% dimethyl sulfoxide (DMSO) in phosphate-buffered saline. This was incubated for 1 min at room temperature, the DMSO was aspirated, and 10 ml of the appropriate medium was added to the dish. The cells were incubated at 37°C in 5% $CO_2$ for several days, and samples of medium were taken at various times for assay as described in the text.

The procedure used for batch transfections was similar to that described above for 10-cm dishes. Approximately $3 \times 10^6$ cells were plated on a 25-cm tissue culture dish (Nunc, Roskilde, Denmark) and allowed to grow for 3 days. The desired amount of DNA in 360 μl of Tris-buffered saline was added to 720 μl of warm DEAE-dextran, and this mixture was added to the cells in a total volume of 36 ml of Nuserum. A 30-ml portion of 10% DMSO in phosphate-buffered saline was used to shock the cells for 1 min at room temperature. After incubation in 60 ml of medium for 24 h, the cells were trypsinized and split equally into six 10-cm dishes, each of which contained 10 ml of medium.

**hGH assay.** hGH levels in the medium were measured with a solid-phase two-site radioimmunoassay kit under the conditions recommended by the manufacturer (Hybritech Inc.). This assay can detect as little as 0.2 ng of hGH per ml and is linear in the range of 0.2 to 50 ng/ml. When necessary, samples with concentrations of hGH in excess of 50 ng/ml were diluted into the linear range of the assay with horse serum.

**CAT assay and RNA analyses.** CAT assays were performed as described previously (8). Total RNA was prepared from tissue culture cells, and Northern blot analysis was carried out with 1.2% agarose–formaldehyde gels as described previously. The resulting nitrocellulose filter was prehybridized overnight and then hybridized to a $^{32}P$-labeled hGH cDNA (13) probe ($3 \times 10^6$ cpm of a $5 \times 10^8$-cpm/μg nick-translated probe) in 6 ml containing 50% formamide and 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) overnight at 42°C (12). The filter was washed for 30 min in 2× SSC–0.1% sodium dodecyl sulfate at room temperature followed by 30 min in 0.4× SSC–0.1% sodium dodecyl sulfate at room temperature and then exposed to X-ray film.

## RESULTS

A series of plasmids were constructed which contain the hGH gene and utilize hGH expression and secretion as a measure of promoter strength and for defining factors affecting regulation of eucaryotic promoters (Fig. 1) as detailed in the Materials and Methods section. The plasmid vector p0GH contains no eucaryotic promoter and is designed for measuring expression directed by an inserted promoter; pTKGH contains the herpes simplex virus TK promoter and is designed to test for enhancer effects of inserted DNA fragments; and pXGH5 contains the mMT-I promoter and is useful for high-level, regulated expression of hGH in a variety of cell types (Fig. 1). These plasmids direct low, moderate, and high levels of hGH expression, respectively (see below).

An initial characterization of the hGH transient expression system included: (i) a time course of hGH accumulation in the medium of transfected cells; (ii) the dose-response relationship between input DNA and expressed mRNA and secreted protein; (iii) the sensitivity of the system; (iv) the utility of the system for the study of regulation of gene expression; and (v) the adaptability of the system to various cell types and transfection protocols. The first four of these parameters were examined by using the DEAE-dextran transfection protocol to introduce pXGH5, p0GH, and pTKGH into L cells, a mouse fibroblast line. In these experiments, the levels of hGH secreted into the medium were measured with a commercially available (Hybritech) radioimmunoassay in which the amount of hGH specifically bound by a first monoclonal antibody on a solid support is measured with a second, $^{125}I$-labeled monoclonal antibody directed against a different hGH epitope. This sandwich assay can detect as little as 0.2 ng of hGH per ml and is linear in the range of 0.2 to 50 ng/ml.

**Time course of hGH accumulation.** After transfection of L cells with the plasmid pXGH5 by the DEAE-dextran method, the expression of hGH was first detectable at approximately 24 h (Fig. 2). Total accumulation of secreted hGH increased rapidly for approximately 3 days (Fig. 2). During the next week, the amount of hGH produced per day remained relatively constant, but from day 11 on, hGH synthesis and secretion gradually diminished (data not shown). Comparison of rates of synthesis with levels of accumulation demonstrated that the secreted hGH is stable in the medium (data not shown). Typically, hGH expression stopped within 3 weeks, although on some occasions it persisted for more than 6 weeks. Extended expression was seen for some, but not all, cell types with hGH-expressing plasmids (see below) and previously has been described for CAT-expressing plasmids transfected into the human cell line 293 (2). This long-term expression presumably reflects the achievement of a stable chromatin state in the transfected cells, as has been shown for CAT-expressing plasmids in both calcium phosphate- and DEAE-dextran-transfected monkey CV-1 cells (17).

To determine the amount of hGH stored in the cells relative to the amount secreted, we measured cellular hGH levels 2 and 4 days after transfection with various amounts of pXGH5. The transfected cells were washed twice with phosphate-buffered saline osmotically swelled (2 mM $MgCl_2$, 20 mM Tris, pH 7.5), and lysed (0.1% Triton, 20 mM Tris, pH 7.5). The lysate was collected, centrifuged to remove

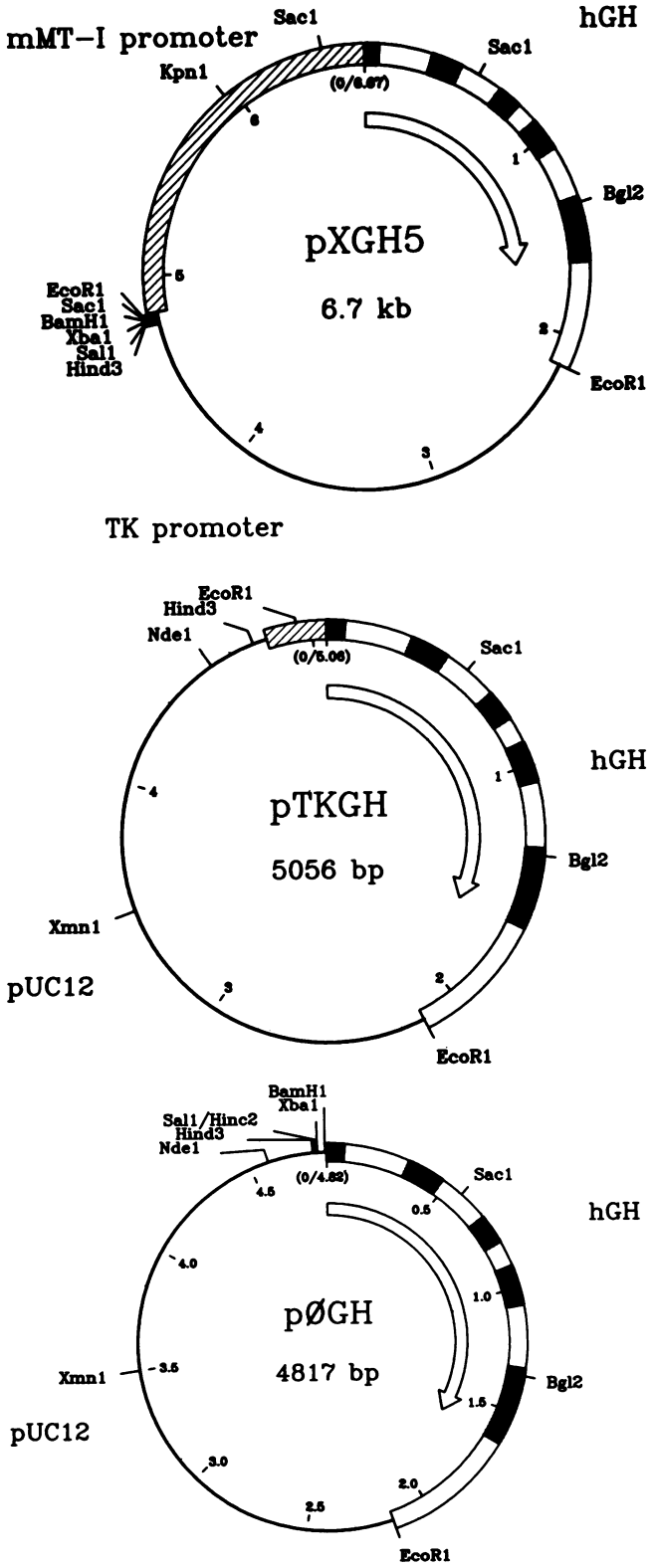


FIG. 1. Restriction maps of hGH expression vectors. The sites for all commercially available restriction enzymes which have one site in p0GH and pTKGH (except *Eco*RI which cuts pTKGH twice) are indicated. A limited number of enzymes which are known to cut pXGH5 are shown. p0GH has no eucaryotic promoter. pTKGH and pXGH5 have the herpes simplex virus TK promoter and the mMT-I promoter, respectively, fused to the hGH structural gene (see Materials and Methods for details on plasmid constructions). The arrows indicate the predicted primary transcripts of the hGH gene, open boxes indicate introns or 3'-flanking regions in the hGH gene, filled boxes indicate hGH exons, hatched boxes indicate promoter fragments, and solid lines indicate pUC12 plasmid vector sequences. kb, Kilobases; bp, base pairs.

cellular debris, and assayed for hGH. Two days after transfection, approximately 1.5% of the total hGH was present inside the cells, and 4 days after transfection, approximately 0.5% was present inside the cells. These values are consistent with results obtained for cells with nonclassical (constitutive) secretory pathways (15) and suggest that the L cells do not store the newly synthesized hGH. We have since transiently transfected L cells with various fusion genes and produced large quantities of several secreted proteins, including the variant hGH, mutant hGHs, and human proinsulin (data not shown).

The very rapid rate of hGH accumulation in the medium during the first few days after the transfection as well as the persistence of hGH expression suggested that the pXGH5 DNA was replicating in the transfected cells. To investigate this possibility, low-molecular-weight DNA was purified from nuclei of transfected cells by Hirt extraction (10) and digested with the restriction enzymes *Sau*3A, *Dpn*I, and *Mbo*I. The products were resolved by electrophoresis on 1% agarose gels, blotted, hybridized to a $^{32}$P-labeled hGH cDNA probe, and visualized by autoradiography (data not shown). At days 2, 5, and 8 posttransfection, the transfected DNA was resistant to cleavage with *Mbo*I and sensitive to *Dpn*I and *Sau*3A, indicating that it retained a bacterial pattern of methylation and had not been replicated. In addition, hybridization of the Hirt-extracted DNA to the hGH cDNA probe showed that the nuclear levels of pXGH5 DNA decrease rapidly during day 1 after transfection, but then remain relatively constant for approximately 8 days thereafter. This situation is very similar to those previously described (2, 17).

**Dose-response studies.** The relationships between the amount of transfected pXGH5 or pTKGH DNA and the level of hGH expression in L cells are shown in Fig. 3. The amount of secreted hGH accumulated in the medium is proportional to the amount of transfected pXGH5 DNA in the range of 10 ng to 4 μg; from 4 to 10 μg, the slope of the curve decreases, and above about 15 μg of input DNA the expression actually decreases with increasing DNA added. The amount of hGH secreted after transfections with pTKGH is also linear up to approximately 4 μg of input DNA but decreases dramatically when amounts of pTKGH in excess of 4 μg are used. It is worth noting that the ratio of hGH expression, and hence presumably the relative strengths of the mMT-I and TK promoters, is approximately 3 to 1 within the linear hGH expression range, but varies between 4 to 1 and 25 to 1 at higher concentrations of input DNA. Interpretation of differences in expression with the hGH transient expression system or any other system, for that matter, must only be made after due consideration is given to such dose-response data. Other experiments (data not shown) indicate that the level of p0GH expression was approximately 0.5% of pXGH5 expression and that the addition of pUC12 carrier DNA had no effect on either pXGH5 or pTKGH transient expression levels. Finally, we observed differences of up to threefold in transient expression levels of a given plasmid between experiments with the DEAE-dextran protocol. This variability is much less than that observed with the calcium phosphate transfection technique and may be due to small differences in cell viability and in the quality of DNA precipitates between experiments.

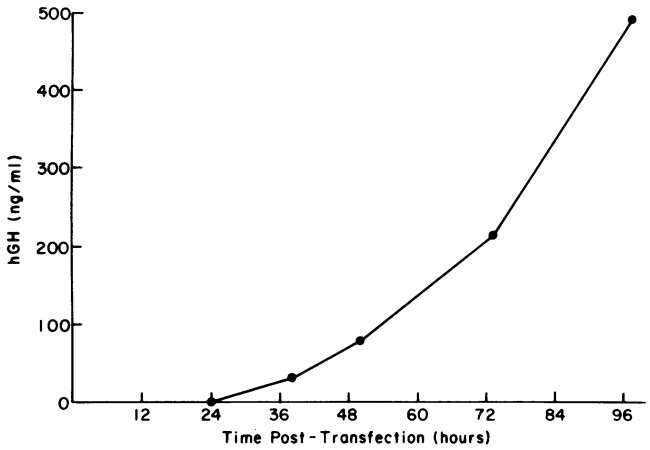


FIG. 2. Time course of hGH expression. Approximately $5 \times 10^5$ L cells were transfected with 1 μg of pXGH5 by using DEAE-dextran, followed by DMSO shock. At various times after DMSO shock, samples of medium were assayed for hGH. The medium was not changed during the course of the experiment, and therefore hGH levels shown are the total accumulation in the medium. Values of hGH expression shown are averages of three plates transfected in parallel: 38-h time point (30.6 ng of hGH per ml ± standard deviation of 1.2 ng/ml); 50 h (79.1 ± 1.3); 73 h (216.8 ± 12.6); 97 h (499.1 ± 18.5).

**RNA studies.** To determine the size of the mMT-I–hGH fusion mRNA, L cells were transfected with pXGH5 and harvested 2 days later for the preparation of cytoplasmic RNA. RNA samples were separated by electrophoresis on 1.2% agarose–formaldehyde denaturing gels, transferred to nitrocellulose filters, and hybridized to an hGH cDNA probe. Figure 4 shows the resulting autoradiograph when RNA samples derived from plates transfected with 0, 0.25, 0.5, and 1 μg (lanes 1 to 4, respectively) of pXGH5 were analyzed. The approximately 900-nucleotide hybridizing band present in lanes 2 to 4 is the expected size for the mature mRNA derived from the mMT-I–hGH fusion gene, which is predicted to contain 64 nucleotides of mMT-I sequences and 817 nucleotides of hGH sequences, in addition to a poly(A) tail. Furthermore, the intensity of the fusion RNA band is roughly proportional to the amount of transfected pXGH5 DNA. In combination with the dose-response data presented above, this result suggests that the amount of hGH secreted into the medium accurately reflects the amount of the fusion gene RNA present in the cell. Last, for all concentrations of input DNA, the amount of secreted protein is directly proportional to the amount of fusion gene mRNA present in the cells, indicating that secretion is not a rate-limiting step in hGH expression.

**Sensitivity studies.** To directly compare the sensitivities of the hGH and CAT transient assay systems, equal amounts of pTKGH and pTKCAT, two plasmids with identical TK promoter sequences, were cotransfected into L cells. Two days after the transfection, a sample of medium was collected for the hGH assay, and the cells were harvested for the CAT assay. Table 1 shows that the expression of 10 ng of input pTKGH DNA can be detected by the hGH radioimmunoassay, whereas at least 100 ng of input pTKCAT is required to detect expression by the CAT assay. This indicates that the hGH transient assay is at least 10-fold more sensitive than the CAT assay with these particular constructs in L cells. Presumably, the hGH transient technique could be made even more sensitive when it is considered that only 1% of the available medium was sufficient to detect a significant signal in the hGH assay whereas 25% of the harvested cells were used for the CAT assay. We estimate that as little as 2 to 4 ng of pTKGH could be readily detected by the hGH transient system, and we in fact were able to detect expression from as little as 1 ng of pXGH5.

**Regulation.** The utility of these vectors for studying regulatory effects on promoter activity was demonstrated in two different ways. First, the levels of transiently expressed hGH were measured with or without the inclusion of the SV40 enhancer into the region upstream of the TK promoter in pTKGH. pTKGH and its enhancer-containing derivative, pSVTKGH, were introduced in XC cells, a rat fibroblast line, and secreted hGH levels were determined. The inclusion of the SV40 enhancer caused a 35- to 60-fold increase in expression of hGH in transfected rat XC cells (Fig. 5A). The SV40 enhancer stimulates its own promoter by 50- to 100-fold in the same cell line as measured by CAT activity (P. R. Larsen, personal communication).

The second test of the suitability of the hGH transient expression system for studies on gene regulation was to determine the effects of heavy metals on pXGH5 expression. This plasmid contains the heavy-metal-inducible mMT-I promoter (9). Heavy metals such as zinc are toxic to cells, and this toxicity must be taken into account when measuring the effects of zinc on the expression of specific genes. The addition of 100 μM $ZnSO_4$ to the medium of L cells transfected in batch (see Materials and Methods) with pXGH5 caused a 20% decrease in the accumulation of hGH as compared with that in medium lacking zinc (Fig. 5B). It is necessary to normalize this effect to that of a non-zinc-regulated promoter since, as mentioned above, a high dose of zinc is toxic to cells. When pTKGH was batch transfected into L cells that were subsequently grown in the presence of 100 μM $ZnSO_4$, a 13- to 26-fold decrease in secreted hGH was observed as compared with growth in zinc-free medium (Fig. 5B). Taken together, these results indicate that 100 μM $ZnSO_4$ causes a 10- to 20-fold increase in pXGH5 expression (Fig. 5B, insert). The level of this effect is similar to the approximately 15-fold RNA induction caused by zinc on metallothionein-TK fusions transfected into BHK cells (18).

**Other cell types.** In addition to mouse L and rat XC cells, all other cell types examined so far were able to express

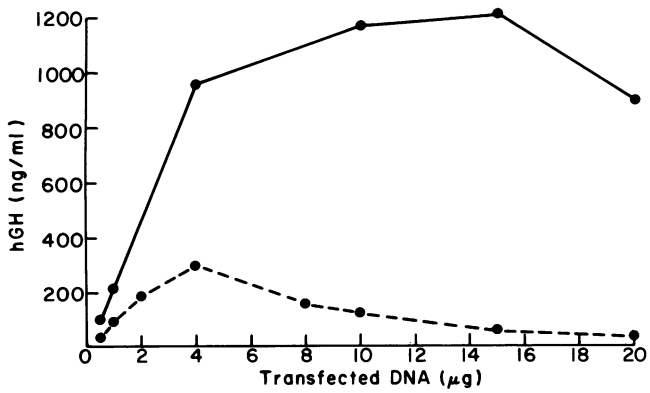


FIG. 3. Dose-response curve of hGH expression versus amount of input DNA. Approximately $5 \times 10^5$ L cells per plate were transfected with the indicated amounts of either pXGH5 (solid line) or pTKGH (dashed line) DNA. Ninety-six hours after removal of DNA and DMSO shock, the medium was assayed for hGH. Amounts of hGH expression represent averages of three plates transfected in parallel.

significant amounts of hGH after transfection with pXGH5. These include the rat pituitary cell lines, GC and $GH_4$; the mouse pituitary cell line, AtT-20; the human choriocarcinoma cell line, JEG; the monkey kidney cell line, CV-1; and rat primary pituitary cells. As expected, the levels of hGH expression were strongly influenced by the same factors which affected the efficiency of expression of CAT from appropriate vectors. For example, GC cells could not be transfected efficiently with pXGH5 or pTKCAT with the DEAE-dextran protocol but expressed reasonable levels of hGH and CAT, respectively, after transfection with a mild calcium phosphate treatment protocol. Similarly, optimal concentrations of DEAE-dextran varied with cell type, as did the time of onset of hGH secretion (data not shown).

## DISCUSSION

We developed a new series of vectors for the analysis of regulation of eucaryotic gene expression based on the secretion of hGH from transiently transfected cells in culture. This approach offers several advantages over generally utilized transient expression systems. In particular, the hGH system is different from those previously described in an important qualitative way: it is not necessary to destroy the cells to assay them for expression. In principle, any secreted peptide hormone such as human insulin (15) or bovine growth hormone (16) could be used in a similar fashion, but as described they are of limited use owing to protein instability in the medium (human insulin) and to the relatively cumbersome protein assays currently available (human insulin, bovine growth hormone).

The properties of the hGH system allow experiments to be done that require many assay points, such as kinetic analysis, which would be difficult or impossible with assays that require a separate plate or flask for each assay point. For example, it should be possible to accurately measure a steady-state rate of expression for a single transfected plate and then to determine the effect of an inducer on the level of expression from the same plate of cells. Futhermore, a relatively large number of cells can be transfected in batch, divided into equal aliquots, and used to measure the effects of a variety of factors on a series of identically transfected cells. This batch-transfection protocol, which was followed for the heavy-metal induction of pXGH5 expression (Fig. 5B), circumvents the problem of experiment-to-experiment variability, which would otherwise make comparing the levels of expression from independent transfections difficult.

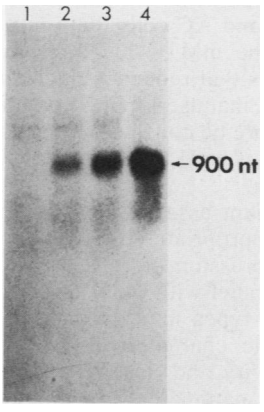


FIG. 4. Northern blot of hGH mRNA synthesized by pXGH5. L cells were transfected with various amounts of pXGH5, and total cytoplasmic RNA was prepared (1) from one plate 2 days after removal of DNA and DMSO shock, resolved by electrophoresis through 1.2% agarose–formaldehyde gels, and blotted onto a nitrocellulose filter. hGH mRNA was visualized by autoradiography after hybridization with a $^{32}$P-labeled hGH cDNA probe. The size of the hGH mRNA was estimated relative to stained rRNA markers in an adjacent lane (data not shown). Input pXGH5: lane 1, 0; lane 2, 250 ng; lane 3, 500 ng; and lane 4, 1 μg. nt, Nucleotides.

TABLE 1. Relative sensitivity of hGH and CAT assays[a]

| DNA (μg) | Secreted HGH | | | Acetylated chloramphenicol | | |
|---|---|---|---|---|---|---|
| | cpm | Corrected cpm | ng/ml ± SD | cpm | Corrected cpm | % Conversion |
| 1 | 13,599 | 12,352 | 48.8 ± 0.4 | 16,450 | 15,439 | 7.5 |
| 0.1 | 2,090 | 843 | 3.4 ± 0.1 | 1,678 | 667 | 0.8 |
| 0.01 | 1,348 | 101 | 0.4 ± 0.02 | 807 | (−204) | (0.0) |

[a] The indicated amounts of pTKGH and pTKCAT, a CAT expression vector which has the same TK promoter as pTKGH (E. Proust and D. D. Moore, Gene, in press), were transfected into L cells. At 48 h after removal of DNA and DMSO shock, a sample of the medium (0.1 ml of 10 ml total) was assayed for hGH levels, and a sample of the cell extract (50 μl of 200 μl total) was assayed for CAT. The resultant counts per minute of either $^{125}$I-labeled anti-hGH antibody bound to the solid support or acetylated [$^{14}$C]chloramphenicol are listed, along with conversions to nanograms of hGH and percent conversion to acetylated forms, respectively. For secreted hGH, corrected counts per minute = total counts per minute − background counts per minute. The hGH assay background equals 1,247 cpm and consists of the intrinsic background of the assay (551 cpm) and background owing to 10% calf serum (696 cpm). Standard deviation is based on three sample points. For acetylated chloramphenicol, corrected counts per minute = total counts per minute − background counts per minute. The CAT assay background equals 1,011 cpm (from a CAT assay with no cell extract).

The hGH transient expression system is at least 10-fold more sensitive than the CAT system as shown by the amount of signal detected after transfections of L cells with various levels of pTKGH and pTKCAT. The hGH transient expression system is therefore much more sensitive than other systems such as those based on the expression of β-galactosidase (3) and xanthine-guanine phosphoribosyl transferase (4). Calculations of the total amounts of hGH and CAT produced in such transfections, based on the known specific activity of bacterially synthesized CAT (19), indicate that this sensitivity difference is due to an approximately 100-fold-greater accumulation of hGH protein than CAT protein. This surprising bias in favor of hGH production could be the result of the relative instability of the CAT nuclear or cytoplasmic mRNA, the inactivation or instability of intracellular CAT versus extracellular hGH, or to a combination of these and other factors. Preliminary experiments designed to study these possibilities suggest that cytoplasmic levels of CAT mRNA are much lower than those of hGH mRNA when equivalent amounts of the two constructs are transfected into L cells (data not shown). It is emphasized that the greater sensitivity of the hGH transient expression system as compared with the CAT system has been demonstrated only in mouse L cells. Presumably, both the cell type and specific fusion genes under study have significant influences on the sensitivity of whatever assay system is being used, and these factors must be taken into account.

The hGH transient expression system is well suited for use as an internal control for normalizing the efficiency of expression between different transfections of CAT-expressing plasmids. The sensitivity of the hGH system enables hGH levels to be measured after transfection with a very small amount of plasmid, thus avoiding potential problems of competition between control and experimental expression units. It is clear from the dose-response curves

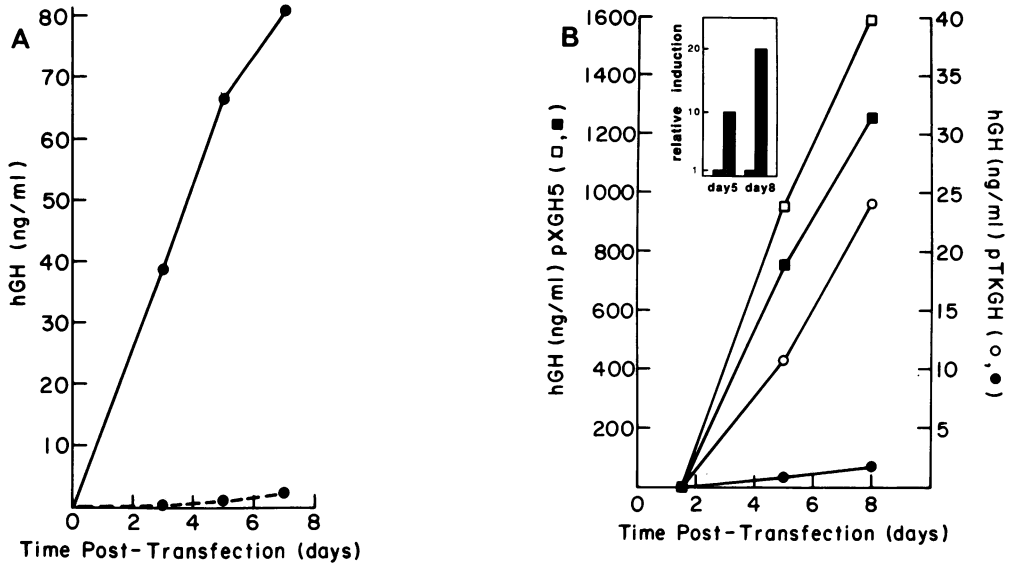


FIG. 5. Utility of hGH transient expression system for the study of regulation of gene expression. (A) Effects of the SV40 enhancer on pTKGH expression in XC cells. Approximately $5 \times 10^5$ XC cells per plate were transfected with 1 μg of pSVTKGH (——), a derivative of pTKGH which contains the SV40 transcriptional enhancer upstream of the TK promoter, or 1 μg of pTKGH (----). Levels of hGH expressed after transfection were measured at the indicated times after removal of DNA and DMSO shock. Amounts of hGH expression represent the average of three plates transfected in parallel. (B) Effects of zinc on pXGH5 expression in L cells. Approximately $3 \times 10^6$ L cells were transfected in batch (see Materials and Methods) with 6 μg of pXGH5 or pTKGH and trypsinized and replated ($5 \times 10^5$ cells per plate) the following day. Cells from three of these plates were grown in the presence of 100 μM $ZnSO_4$, and cells from three plates were grown in the absence of $ZnSO_4$. Levels of hGH expressed after transfection were measured at the indicated times after removal of DNA and DMSO shock. Symbols: □, pXGH5, no $ZnSO_4$; ■, pXGH5, 100 μM $ZnSO_4$; ○, pTKGH, no $ZnSO_4$; ●, pTKGH, 100 μM $ZnSO_4$. Insert: Relative induction of 100 μM $ZnSO_4$ on pXGH5 as normalized to pTKGH. Black bars, pTKGH; gray bars, pXGH5.

presented above that the levels of transient expression can become unpredictable when large quantities of DNA are transfected into cells (Fig. 3). The use of relatively large quantities of a control plasmid (as is frequently needed with, for example, Rous sarcoma virus-β-galactosidase control plasmids [6]) can have a significant effect on expression of the experimental gene, which in turn might distort interpretation of the results. The ability to use relatively small quantities of pXGH5 to normalize transfection efficiencies should minimize this problem of DNA dose effects. The fact that the hGH assay uses secreted products provides a means of avoiding competitive demands on the use of the same materials, such as preparation of RNA and active enzyme from the same extract. Since the hGH assay is simple and rapid (4 h), it is possible to assay the medium before initiating the rest of the experiment to determine whether appropriate transfection efficiency has been achieved to warrant further experimentation. The hGH transient expression system has provided an effective internal control for CAT expression in assays of regulatory effects on a single promoter (P. R. Larsen, personal communication) and on a series of promoter mutants and *trans*-activation studies (10a).

One potential disadvantage to the hGH transient expression system is the possibility that the secreted hGH itself will interfere with gene expression through an autocrine mechanism, although no obvious effects of hGH on expression have been observed with the cell lines tested so far. In principle, such effects could be obviated by inclusion of a growth hormone in the medium which is biologically active but does not cross-react with the monoclonal antibodies used in the hGH radioimmunoassay. A more direct approach, currently under development, is to render the secreted hGH biologically inactive, without affecting its binding to the antibodies used in the assay. Like CAT and other enzymatic systems for measurement of promoter activity, the hGH system is clearly not a direct reflection of the rate of transcriptional initiation. The additional processes involved in secretion might, in some cell types, make the hGH assay a somewhat less reliable indicator of transcription. We studied several regulatory effects on pXGH5 expression in a line of a typical secretory cell, the rat pituitary AtT-20 line, and observed no differences in the regulated levels of secreted hGH in comparison with nonsecretory cells such as L cells, CV-1 cells, and XC cells (data not shown). Last, we determined that the mMT-I–hGH fusion gene in pXGH5 contains sequences that render it glucocorticoid responsive (R. F Selden et al., manuscript in preparation). This intrinsic effect must therefore be considered when the hGH transient expression system is used to study glucocorticoid regulation of gene expression.

The hGH transient assay system is sensitive, simple to perform, and is appropriate either for the analysis of regulation of gene expression or as a control for efficiency of transfection in parallel with other transient expression protocols. Other cell types may differ from L cells, used for much of the basic characterization of the system, with respect to sensitivity and dose-response relationships, for example. If these parameters are monitored for each cell type to be studied, however, we believe that the hGH transient assay system will have a broad range of applications to molecular biology.

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