Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 8283–8287, November 1986
Genetics

Repression mediates cell-type-specific expression of the rat growth hormone gene

(transcriptional silencer/chloramphenicol acetyltransferase assay/transfection/gene regulation)

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Communicated by Eugene Braunwald, July 17, 1986

ABSTRACT **A plasmid containing 1.8 kilobase pairs of rat growth hormone (rGH) promoter and upstream flanking sequences fused to the bacterial gene for chloramphenicol acetyltransferase (CAT) was transiently introduced into pituitary, fibroblast, and kidney cell lines. Significant CAT activity was detectable only in the pituitary cell lines, demonstrating that this relatively large fragment directs strongly cell-type-specific expression. However, plasmids containing only 200–300 bases of rGH promoter and flanking sequences directed expression of CAT in all three cell types, suggesting that upstream sequences directly repress the activity of a minimal rGH promoter in nonpituitary cell types. S1 nuclease analysis showed that the RNA synthesis directed by one of the short rGH promoter fragments in fibroblasts initiated from the site used by the natural promoter in pituitary cells. Insertion of rGH upstream sequences in their natural orientation upstream of the mouse metallothionein I promoter caused a decrease in its activity in fibroblasts by a factor of 4, but there was a 2.5-fold increase in its activity in pituitary cells. Insertion of the rGH fragment upstream of the thymidine kinase promoter in either orientation lowered its activity in both fibroblasts and pituitary cells. Thus, the negatively acting rGH flanking sequences can act on a heterologous promoter and have at least some of the properties of positively acting enhancers.**

One of the most basic questions in the molecular biology of higher organisms is the nature of the control mechanisms that direct a given gene to be actively expressed in one cell type and silent in another. The growth hormone gene provides a particularly dramatic example of such cell-type specificity of gene expression: rat pituitary cells in culture can produce at least 10^8-fold more growth hormone than similarly treated rat liver cells (1). It is likely that such a large difference could only be achieved by the concerted action of regulatory effects at several levels.

Cell fusion experiments have suggested that negative factors are involved in at least one such level. For example, when a pituitary cell is fused to a relatively nondifferentiated cell, rat growth hormone (rGH) expression is repressed (2). Such extinction of expression is commonly observed when highly differentiated cells are fused to nondifferentiated cells (reviewed in ref. 3). Biochemical studies of expressed and quiet genes have also associated several general aspects of chromatin structure with negative effects on gene expression (reviewed in ref. 4).

In contrast to these negative effects, examples of positive regulation have been provided by the discovery of sequences termed tissue- or cell-type-specific enhancers (5–9). These elements are located within or near a number of genes expressed in a cell-type-specific manner and act to increase their rate of transcription, but they are active only in appropriate cell types. In at least some cases, specific sequences within the promoters of such genes are also involved in increasing the effectiveness of the positively acting enhancers (9–11).

We describe here specific sequence elements that are involved in cell-type-dependent repression of gene expression. We show that sequences upstream of the rGH promoter strongly repress its activity in fibroblasts and kidney cells, but not pituitary cells. These rGH flanking sequences also show repressive effects when placed upstream of the heterologous mouse metallothionein I or herpes simplex virus thymidine kinase (TK) promoters. These negative elements have properties similar to those of both the negatively acting yeast elements, termed silencers (12), and the positively acting enhancer elements.

MATERIALS AND METHODS

Plasmid Constructions. Standard techniques for DNA manipulations were used for all plasmid constructions (13). pRGH1753 was constructed by converting a 1766-base-pair (bp) *Eco*RI/*Xho* I fragment from the rGH gene (14) to a *Bam*HI fragment using linkers and inserting it into the *Bam*HI site of pOCAT1, a promoter insertion chloramphenicol acetyltransferase (CAT) expression vector (15). pRGH237 and pRGH183 were constructed by inserting *Bgl* II to *Bam*HI and *Sau*3A to *Bam*HI fragments, respectively, from pRGH1753 into pOCAT1. pRGH309 was constructed by conversion of the *Kpn* I site of pRGH1753 into a *Bam*HI site and insertion of a resultant *Bam*HI fragment into pOCAT1. The deletion derivatives of pRGH1753 were created by cutting with either *Kpn* I or *Bst*XI, which do not cleave the CAT vector, treatment with T4 DNA polymerase to generate flush ends in the case of the *Bst*XI deletions, and religation. *Kpn* I cleaves the rGH fragment at positions −1198 and −309. *Bst*XI cleaves at −1236, −554, and −210. The plasmid pRGH-1753ΔBst-1, which is missing only the furthest upstream fragment of the two internal *Bst*XI fragments presumably was a result of partial initial *Bst*XI cleavage. pUTKATRGH1 and pUTKATRGH2 were constructed by insertion of the *Bgl* II fragment of pRGH1753 in either orientation into the *Bam*HI site of pUTKAT1, a CAT expression vector designed for insertion of regulatory elements near the TK promoter (14). pUTKATRGH1ΔBst was created by deletion of the internal *Bst*XI fragment from the rGH fragment contained in pUTKATRGH1. pXGHRGH1 and pXGHRGH2 were constructed by inserting the same rGH *Bgl* II fragment into the *Bam*HI site of pXGH103, a deletion derivative of the plasmid pXGH5 (16) in which a 210-bp *Sac* I to *Bgl* II fragment

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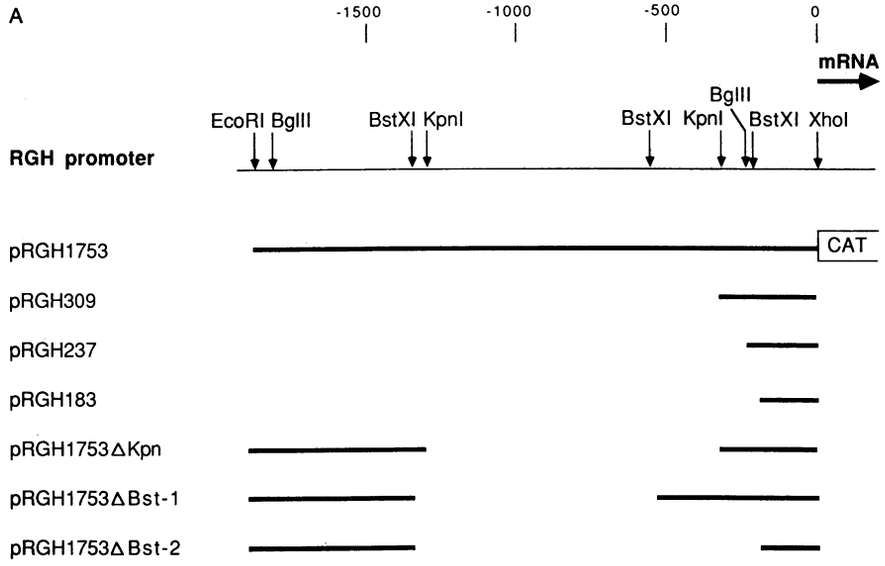
Abbreviations: rGH, rat growth hormone; hGH, human growth hormone; TK, thymidine kinase; CAT, chloramphenicol acetyltransferase; bp, base pair(s).

containing the mouse metallothionein I promoter directs expression of human growth hormone. The *Bam*HI site used to insert the rGH fragment is immediately upstream of the mouse metallothionein I promoter fragment. pXGH103 was kindly supplied by Richard Selden.

Transfections and Expression Assays. LTK$^-$ and XC cells were transfected by using DEAE/dextran essentially as described by Lopata *et al.* (17), including a 2-min shock with 10% dimethyl sulfoxide. GC, GH_4C1 (kindly supplied by Armen Tashjian), and CV-1 cells were transfected by using $CaPO_4$ essentially as described (18). Cells were harvested with trypsin/EDTA, washed, and lysed by resuspension in 0.2 ml of 0.25 M Tris·HCl (pH 7.8) and 0.5% Triton X-100. Ten to 100 μl of the 10,000 × *g* supernate (50–120 μg of protein) was assayed for CAT activity according to the protocol of Gorman *et al.* (19), except that acetyl coenzyme A concentrations were increased to 8 mM and [^{14}C]chloramphenicol concentrations were lowered to 30 μM (0.2 μCi per reaction mixture; 1 Ci = 37 GBq). GC and GH_4C1 extracts were heated to 70°C for 15 min before assay to inactivate an uncharacterized inhibitor. Human growth hormone (hGH) expression directed by the control plasmid pXGH5 (16), a fusion of the mouse metallothionein I promoter and the hGH structural gene, or by pXGH103 and its derivatives, was measured in media by a solid-phase immunoradiometric assay (Hybritech, San Diego, CA). Variability between transfections was minimized by normalization of CAT assay results (in % conversion to acetylated derivatives per 4 hr) to amount of protein in the cell extracts (Bio-Rad) and to levels of hGH expressed by a constant amount of cotransfected pXGH5. CAT/hGH ratios were computed as 1000 × {[(CAT activity)/(4-hr assay) × (100 μg of cell extract)]/(cpm of specifically bound hGH)}.

RNA preparation and S1 nuclease analysis were performed basically as described (20). The S1 nuclease probe was prepared by extension of a 5′ ^{32}P-end-labeled oligonucleotide, 5′ CAACGGTGGTATATCCAGTG 3′, which is complementary to mRNA sequences encoding amino acids 5–12 of the CAT protein. Denatured pRGH237 DNA was used as the template for probe extension. The extended probe was

A



B

	RELATIVE EXPRESSION			SPECIFICITY	
PLASMID	GC CELLS	LTK$^-$ CELLS	CV-1 CELLS	GC/LTK$^-$	GC/CV-1
pUTKAT1	100	100	100	1	1
pRGH1753	71	0.4	<2	175	>35
pRGH309	184	9.4	92	20	2
pRGH237	200	45	1400	4	0.14
pRGH183	100	12	110	8	1
pRGH1753ΔBst-1	317	0.4	3	790	106
pRGH1753ΔBst-2	36	4.0	7	9	5

FIG. 1. (*A*) Plasmids containing rGH promoter and 5′ flanking regions fused to the CAT gene. A restriction map of rGH promoter and 5′ flanking sequences (thin line) is shown, with a scale in base pairs. Heavy lines indicate rGH sequences that are present in the various plasmids. Plasmids are named by the amount of rGH sequences 5′ to the start site of rGH transcription in pituitary cells that they contain, except for the internally deleted derivatives of pRGH1753. (*B*) Influence of elements in the 5′ flanking region on relative CAT activity directed by rGH promoter fragments in various cell lines. Results of CAT/hGH ratios for equivalent molar amounts of each plasmid have been normalized to the expression from 10 μg of pUTKAT1. For GC and LTK$^-$ cells, CAT activity measured for pUTKAT1 was similar to that described in Table 1. For CV-1 cells, measured pUTKAT1 CAT activity represented ≈4% conversion to acetylated forms, corresponding to ≈10% conversion/[(4-hr assay) × (1 mg of cell extract)]. Results presented are the mean of two to eight such experiments, each consisting of parallel triplicate transfections.

cut at the *Bgl* II site at position −237 in the rGH 5′ flanking region and purified as described (20).

RESULTS

To determine which sequences were responsible for the strong cell-type specificity of expression of rGH, a series of plasmids was constructed containing variable amounts of the rGH promoter and 5′ flanking sequences fused to a CAT expression unit (15). These plasmids, diagramed in Fig. 1*A*, were transfected into a variety of cell lines, including the rGH-secreting pituitary cell lines GC or GH_4C1, the mouse fibroblast cell line LTK^-, the rat fibroblast cell line XC, and the monkey kidney cell line CV-1. Four days after transfection, the level of CAT activity directed by the promoter constructs was assayed as a monitor of promoter activity. A potentially serious source of error in these and similar experiments, variability in CAT expression due to variation in transfection efficiency, was avoided by cotransfection with a control plasmid that constitutively expressed hGH (16). In all cases, the amount of CAT expression was normalized to the amount of hGH expression.

As shown in Table 1, an ≈1.8-kilobase fragment containing the rGH promoter and upstream sequences directed appropriate pituitary cell-specific expression in such experiments. pRGH1753 (see Fig. 1*A*) directed at least 280 times more CAT activity in the GC pituitary cells than in the LTK^- fibroblasts, when normalized to the level of hGH directed by equivalent amounts of the internal control plasmid pXGH5. An alternative comparison to the level of CAT expression directed by equivalent amounts of the herpes simplex virus TK promoter suggests that the promoter activity of this rGH fragment is 175 times stronger in pituitary cells. In both cases, the very low level of expression directed by the pRGH1753 in the LTK^- cells makes the exact ratio difficult to determine.

In contrast to the high cell-type specificity of expression directed by pRGH1753, the three plasmids containing the shortest rGH promoter fragments, pRGH309, pRGH237, and pRGH183, direct significant levels of CAT expression in fibroblasts and kidney cells as well as in pituitary cells. As shown in Fig. 1*B*, this CAT activity is generally similar to or less than the levels directed by the relatively weak TK promoter. The loss of specificity is most striking for pRGH237, which directs >100-fold more CAT expression than pRGH1753 in fibroblasts, and >700-fold more in CV-1 cells. Relative to the level of CAT directed by the TK promoter, the CAT activity directed by the fragment in pRGH237 is highest in CV-1 cells, intermediate in GC cells, and lowest in LTK^- cells. Two plasmids containing large internal deletions of the rGH flanking sequences in pRGH1753—pRGH1753ΔBst-1 and pRGH1753ΔKpn (LTK^- cells only)—showed relatively low levels of CAT expression in the nonpituitary cells and correspondingly high levels of cell-type specificity. The derivative with the largest deletion, pRGH1753ΔBst-2, showed both lowered expression in GC cells and increased expression in LTK^- cells, resulting in only moderate specificity. When transfected with the various plasmids, the alternative rat pituitary cell line GH_4C1 gave results similar to those obtained with GC cells. Results with the rat fibroblast cell line XC were similar to those obtained with LTK^- cells.

The large relative increase in CAT expression directed by

Table 1. Cell-type-specific expression directed by the rGH promoter and 5′ flanking region

Cell type	Plasmid	μg	Expression (CAT/hGH)
GC (pituitary)	pRGH1753	12	0.39
	pXGH5	5	
	pUTKAT1	10	0.55
	pXGH5	5	
LTK^- (fibroblast)	pRGH1753	12	0.007
	pXGH5	1	
	pUTKAT1	4	0.74
	pXGH5	1	

GC and LTK^- cells were transfected as described. Results presented are the mean of three to six experiments, each consisting of parallel triplicate transfections. In general, variability between triplicate points in a single experiment and CAT/hGH ratios between experiments was <25%, although absolute transfection efficiencies sometimes varied by severalfold between experiments. For GC cells, pUTKAT1 CAT activity represented ≈8% conversion to acetylated forms per 16-hr assay, corresponding to ≈2.5% conversion/[100 μg of cell extract protein)/(4-hr assay)]. hGH levels were ≈7 ng/ml, corresponding to ≈5000 cpm of specifically bound hGH antibody/100 μl of medium. For LTK^- cells, pUTKAT1 CAT activity represented ≈60% conversion to acetylated forms, corresponding to ≈15% conversion/[(100 μg of cell extract protein)/(4-hr assay)]. hGH levels were 50–100 ng/ml, corresponding to 15,000–30,000 cpm of specifically bound antibody per 100 μl of medium. CAT/hGH ratios are computed as 1000 × [(% conversion)/(4 hr) × (100 μg of cell extract protein)]/[(cpm specifically bound antibody)/(100 μl of medium)].

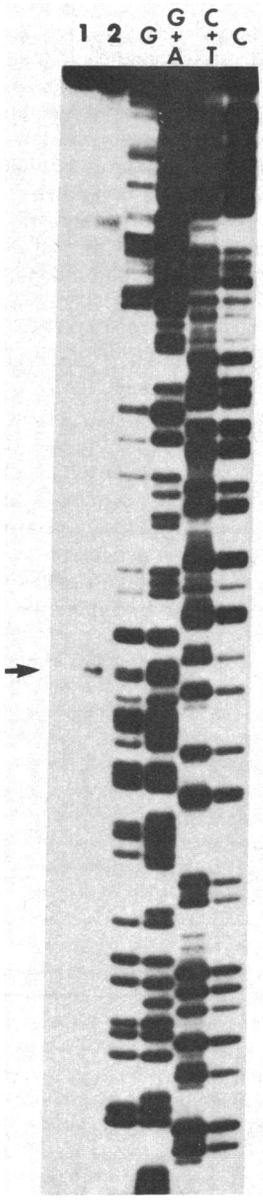


FIG. 2. S1 nuclease analysis of CAT mRNA from transfected LTK^- cells. Total cellular RNA (50 μg) was hybridized overnight to 10^5 cpm of end-labeled probe and treated with S1 nuclease. The probe corresponds to sequences present in the rGH/CAT plasmids used and extends from a 20-bp oligonucleotide complementary to an amino-terminal segment of the CAT coding region to the *Bgl* II site in the rGH promoter. RNA was prepared from cells transfected with either pRGH1753 (lane 1) or pRGH237 (lane 2). Lanes G, G+A, C+T, and C are the result of chemical sequencing reactions on the probe DNA. Arrow indicates position of the 5′ end of authentic rGH mRNA as determined (14).

the shortest rGH fragments in the LTK$^-$ cells suggests that the additional upstream sequences present on the larger fragments act to drastically repress expression from an otherwise functional rGH promoter. Since the 237-bp truncated promoter fragment in pRGH237 generates the highest levels of CAT in both pituitary and nonpituitary cells, such repressive sequences must lie upstream of the *Bgl* II site at −237. The deletion extending from −1236 to −554 in pRGH1753ΔBst-1 has virtually no effect on expression in LTK$^-$ or CV-1 cells. Therefore, this area has no involvement in nonpituitary cell repression, although the slightly increased GC cell expression directed by this plasmid (as well as by pRGH309 and pRGH236) suggests that sequences on the deleted fragment may cause a slight inhibition of expression in pituitary cells. The very low specificity shown by pRGH1753ΔBst-2, which is also missing the sequences from −554 to −210, indicates that at least some repressive elements lie in that interval. Relative to pRGH237, the stepwise decreases in expression directed by pRGH309 and pRGH1753-ΔKpn confirm this localization and suggest that additional negative elements lie upstream of −554.

To test directly whether the difference in CAT expression directed by pRGH237 and pRGH1753 in LTK$^-$ cells was a result of a difference in activity of the rGH promoter, the position of the 5′ end of CAT mRNA directed by the two plasmids was determined by S1 nuclease analysis (Fig. 2). Comparison of protected products to a sequencing ladder generated from the probe fragment shows that cells transfected by the pRGH237 contain CAT mRNA initiated at precisely the position used by the native gene in pituitary cells (14), whereas cells transfected by pRGH1753 do not contain detectable amounts of such RNA. Additional bands of higher molecular weight present in both lanes are generated by either incomplete S1 nuclease cleavage or messages with upstream initiation sites. Such messages should not express CAT because of the presence of several AUG codons just upstream of the appropriate rGH mRNA start (14). We conclude that the expression of CAT directed by pRGH237 in fibroblasts reflects authentic rGH promoter activity.

As an independent test of the repressive activity of the sequences flanking the rGH promoter, a 1450-bp *Bgl* II fragment extending from −1680 to −237 was inserted in either orientation just upstream of the TK promoter in pUTKAT1, a CAT expression vector designed for analysis of transcriptional regulatory elements (15). As shown in Fig. 3*A*, these insertions resulted in significantly lower levels of CAT expression in both LTK$^-$ and GC cells. The drop in expression was larger in the plasmid pUTKATRGH1, in which the rGH fragment is in its normal orientation relative to the promoter, but in both cases it was much lower than the very strong effect these upstream sequences have on their own promoter. Removal of internal rGH sequences from −1236 to −554 by the *Bst*XI-mediated deletion in pUTKATRGH1ΔBst had no effect on expression in GC cells and lowered expression in LTK$^-$ cells slightly. Thus, as expected from the results of the analogous deletion on pRGH1753ΔBst-1, this segment is not responsible for the drop in TK promoter activity. The relative repression in pUTKATRGH1 and its *Bst*XI deletion derivative is slightly stronger in LTK$^-$ cells than in GC cells, resulting in weak (1.5- to 2-fold) apparent cell-type specificity.

These results were extended by inserting the same 1450-bp *Bgl* II fragment into a *Bam*HI site adjacent to the mouse metallothionein I promoter in pXGH103, a plasmid that directs expression of hGH as a reporter protein (Fig. 3*B*). pXGHRGH1, which contains the rGH fragment in its natural orientation, expresses 1/4th as much hGH as pXGH103 in LTK$^-$ cells, and 2.5-fold more hGH in GC cells. Thus, this insertion results in a 10-fold increase in relative promoter activity of the mouse metallothionein I fragment in GC cells as compared to LTK$^-$ cells. The backwards insertion of the rGH fragment in pXGHRGH2 has no effect on hGH expression in LTK$^-$ cells, but it increases hGH expression 5-fold in GC cells. The relative decrease in fibroblast hGH expression directed by pRGHXGH1 confirms that sequences flanking the rGH promoter contain suppressive elements that can act on heterologous promoters. The relative increase in pituitary hGH expression from both insertions suggests that it may also contain positive elements, although similar increases were not seen for the insertions next to the TK promoter.

DISCUSSION

The transient expression studies reported here demonstrate that sequences upstream of the rGH promoter strongly repress its activity in nonpituitary cell types. In the absence of these repressive elements, a number of rGH promoter-containing fragments direct substantially increased levels of CAT expression in fibroblast and kidney cell lines. As shown by S1 nuclease analysis, this CAT expression reflects authentic rGH promoter activity. Deletion mapping of the rGH flanking region shows that at least some of the sequences responsible for cell-type-specific repression lie just upstream of the promoter, between −237 and −554.

A

PLASMID		RELATIVE EXPRESSION GC	RELATIVE EXPRESSION LTK$^-$	SPECIFICITY GC/LTK$^-$
pUTKAT1	TK CAT	100	100	1
pUTKATRGH1	RGH BglII → TK CAT	34	24	1.4
pUTKATRGH2	← TK CAT	52	55	1.0
pUTKATRGH1ΔBst	— → TK CAT	31	16	2.0

B

PLASMID		RELATIVE EXPRESSION GC	RELATIVE EXPRESSION LTK$^-$	SPECIFICITY GC/LTK$^-$
pXGH103	mMTI HGH	100	100	1
pXGH103RGH1	RGH BglII → mMTI HGH	250	26	10
pXGH103RGH2	← mMTI HGH	510	110	4.6

FIG. 3. Influence of elements in the rGH 5′ flanking region on either the TK (*A*) or mouse metallothionein I (mMTI) (*B*) promoter activity in pituitary cells and fibroblasts. Results are expressed as percentage of the parental control (pUTKAT1 or pXGH103), and are the mean of three to six experiments, each including parallel triplicate transfections. In the case of pXGH103 and its derivatives, levels of expression of hGH were normalized to levels of CAT expression directed by constant amounts of pUTKAT1 cotransfected as an internal control.

The promoter activity of the rGH fragments transiently introduced into pituitary cells is consistent with experiments in which similar fragments were fused to an *Escherichia coli* gene and stably introduced into GC cells. The resultant cell lines produced both the bacterial enzyme and an appropriately initiated mRNA (21, 22). The very low level of transient expression directed by pRGH1753 in LTK$^-$ cells is also consistent with two studies in which intact rGH genes with extensive flanking sequences were stably introduced into that cell line. The rGH promoter did not direct any transcription in the resultant transformed cell lines, although aberrant RNA species arising from start sites within the gene were observed (23, 24).

The repressive elements upstream of the rGH promoter can also act on heterologous promoters. Insertion of a relatively large rGH promoter-flanking fragment in its natural orientation upstream of either the herpes TK or mouse metallothionein I promoters results in a decrease in expression in LTK$^-$ cells by a factor of 4. With the TK promoter, a slightly lower repression is also observed in GC cells, resulting in a weak cell-type specificity. Stronger cell-type specificity is observed with the metallothionein I promoter, perhaps reflecting additional action of positive elements within the rGH sequences. In both cases, the relative magnitude of the repressive effect on the heterologous promoter is much lower than the repression of the rGH promoter. It is possible that the rGH promoter contains additional cell-type-specific elements that are particularly responsive to the effects of the upstream sequences.

The mechanism by which the upstream sequences affect promoter activity is unknown. Since they act on different promoters to lower expression of a completely foreign CAT mRNA, a simple interpretation would be that they actively repress transcription initiation. Direct measurements of transcriptional rates would be necessary to conclusively demonstrate this, however.

Although only a limited number of examples of mammalian sequences with inherent repressing activity have been documented (25–29), several observations suggest that regulated repression may be important in controlling cell-type-specific gene expression. In the yeast *Saccharomyces cerevisiae*, for example, the expression of the genes specifying the mating type of a cell is regulated by cis-acting sequences, termed silencers (12). These sequences are located near transcriptionally quiet mating type loci and are required for repression of their activity. At least one of these silencer sequences can strongly repress activity of a heterologous promoter (12). Since the sequences flanking the rGH promoter show both cell-type-specific repressive activity and the ability to confer repression onto a heterologous promoter, it is possible that they contain a mammalian silencer(s).

A less well-understood example of cell-type-specific repression of gene activity is provided by the extinction of the expression of many specialized proteins that is commonly observed when a differentiated cell is fused with a less-differentiated cell (3). Extinction of rGH expression is observed when rat pituitary cells are fused with LTK$^-$ cells (2). Recently, experiments have suggested that extinction of some liver specific genes in hepatoma × fibroblast hybrids is a result of the activity of specific trans-acting loci, called *tse*s (30). The *tse* functions are apparently expressed in the fibroblasts and must be continuously active in order to repress the liver-specific functions. It will be of interest to determine whether the expression directed by the rGH promoter constructs discussed above is subject to extinction. Such a response has recently been demonstrated for transient expression directed by a fragment containing the rat albumin promoter and associated regulatory elements (31). However, previously reported results suggest that in stably transfected cell lines rGH genes linked to the simian virus 40 enhancer may escape extinction (32).

It seems apparent that a variety of mechanisms operating at different levels are involved in cell-type-specific regulation of gene expression. For example, a given gene could have a cell-type-specific repression system, such as that described here, as well as cell-type-specific transcriptional enhancer sequences, such as those described for other genes. These regulatory elements could act in combination to maximize the ratio of expression in appropriate and inappropriate cell types. The huge difference between the level of rGH expression in pituitary and hepatoma cells suggests that such combinatorial regulatory effects could be operating in this case.

We thank Richard Selden, Bob Kingston, and Roger Brent for helpful suggestions and comments on the manuscript, and John Smith for the oligonucleotide. This work was supported by a grant from Hoechst AG.

1. Ivarie, R. D., Schacter, B. S. & O'Farrell, P. (1983) *Mol. Cell. Biol.* **3**, 1460–1467.
2. Strobl, J. S., Dannies, P. S. & Thompson, E. B. (1982) *J. Biol. Chem.* **257**, 6588–6594.
3. Davidson, R. L. (1974) *Annu. Rev. Genet.* **8**, 195–218.
4. Weintraub, H. (1985) *Cell* **42**, 705–711.
5. Gillies, S. D., Morrison, S. L., Oi, V. T. & Tonegawa, S. (1983) *Cell* **33**, 717–728.
6. Banerji, J., Olsen, W. & Schaffner, W. (1983) *Cell* **33**, 729–740.
7. Queen, C. & Baltimore, D. (1983) *Cell* **33**, 741–748.
8. Walker, M. D., Edlund, T., Boulet, A. M. & Rutter, W. J. (1983) *Nature (London)* **306**, 557–561.
9. Edlund, T., Walker, M. D., Barr, P. J. & Rutter, W. J. (1985) *Science* **230**, 912–916.
10. Grosschedl, R. & Baltimore, D. (1985) *Cell* **41**, 885–897.
11. Mason, J. O., Williams, G. T. & Neuberger, M. S. (1985) *Cell* **42**, 479–487.
12. Brand, A. H., Breeden, L., Abraham, J., Sternglanz, R. & Nasmyth, K. (1985) *Cell* **41**, 41–48.
13. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
14. Page, G. S., Smith, S. & Goodman, H. M. (1981) *Nucleic Acids Res.* **9**, 2087–2104.
15. Prost, E. & Moore, D. D. *Gene*, in press.
16. Selden, R. F, Burke-Howie, K., Rowe, M. E., Goodman, H. M. & Moore, D. D. (1986) *Mol. Cell. Biol.* **6**, 3173–3179.
17. Lopata, M., Cleveland, D. & Sollner-Webb, B. (1984) *Nucleic Acids Res.* **12**, 5707–5717.
18. Graham, F. L. & van der Eb, A. J. (1973) *Virology* **52**, 456–467.
19. Gorman, C. M., Moffat, L. F. & Howard, B. H. (1982) *Mol. Cell. Biol.* **2**, 1044–1051.
20. Kingston, R. E., Cowie, A., Morimoto, R. I. & Gwinn, K. A. (1986) *Mol. Cell. Biol.* **6**, 3180–3190.
21. Casanova, J., Copp, R. P., Janocko, L. & Samuels, H. H. (1985) *J. Biol. Chem.* **260**, 11744–11748.
22. Crew, M. D. & Spindler, S. R. (1986) *J. Biol. Chem.* **261**, 5018–5022.
23. Moore, D. D., Walker, M. D., Diamond, D. J., Conkling, M. A. & Goodman, H. M. (1982) *Recent Prog. Horm. Res.* **38**, 197–225.
24. Karin, M., Eberhardt, N. L., Mellon, S. H., Malich, N., Richards, R. I., Slater, E. P., Barta, A., Martial, J. A., Baxter, J. D. & Cathala, G. (1984) *DNA* **3**, 147–155.
25. van der Hoorn, F. A., Muller, V. & Pizer, L. I. (1985) *Mol. Cell. Biol.* **5**, 406–409.
26. Gilmour, R. S., Spandidos, D. A., Vass, J. K., Gow, J. W. & Paul, J. (1984) *EMBO J.* **3**, 1263–1272.
27. Bientz-Tadmor, B., Zakut-Houri, R., Libresco, S., Givol, D. & Oren, M. (1985) *EMBO J.* **4**, 3209–3213.
28. Laimins, L., Holmgren-König, M. & Khoury, G. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 3151–3155.
29. Goodbourn, S., Burstein, H. & Maniatis, T. (1986) *Cell* **45**, 601–610.
30. Killary, A. M. & Fournier, R. E. K. (1984) *Cell* **38**, 523–534.
31. Petit, C., Levilliers, J., Ott, M.-O. & Weiss, M. C. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 2561–2565.
32. Strobl, J. S., Padmanaban, R., Howard, B. H., Wehland, J. & Thompson, E. B. (1984) *DNA* **3**, 41–49.