Selective transcription and DNase I protection of the rat prolactin gene by GH₃ pituitary cell-free extracts

(DNA–protein interactions/cell-specific gene expression)

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ABSTRACT Prolactin (PRL) is a member of the growth hormone gene family that is specifically expressed in the lactotroph cells of the anterior pituitary. Whole cell extracts have been prepared from cultured GH₃ rat pituitary tumor cells to study lactotroph-specific expression of the rat PRL (rPRL) gene in an *in vitro* transcription assay. The human α_1 -globin and Rous sarcoma virus promoters efficiently initiate transcription in both HeLa and GH₃ cell extracts, whereas the rPRL promoter containing 425 base pairs of 5' flanking DNA is active only in GH₃ pituitary cell-free extracts. Transcription of the rPRL gene was reconstituted in HeLa cell extracts by the addition of GH₃ cell extracts. DNase I digestion of the rPRL promoter reveals two protected regions centered at positions –55 (I) and –160 (III) that are GH₃ cell-specific and rPRL promoter-selective. These “footprints” overlie a highly conserved 8-base-pair motif, CCTGAT^{AT}TAA. By contrast, footprint II at position –125 is common to both HeLa and GH₃ cell extracts and overlies a 15-base-pair sequence found in all members of the growth hormone gene family. Thus, GH₃ pituitary cell-free extracts selectively transcribe the rPRL gene and contain cell-specific factors that directly interact with the rPRL promoter. These studies provide useful assays to further identify, purify, and characterize pituitary-specific transcription factors and to address the biochemical mechanisms involved in rPRL gene expression.

The growth hormone (GH) gene family, including GH, prolactin (PRL), and chorionic somatomammotropin (placental lactogen), provides a model system to investigate tissue-specific gene expression because these genes are derived from a common ancestral gene, contain very similar DNA sequences (including the 5' flanking regions), and yet are expressed in different tissues (1). GH and PRL are produced in the somatotroph and lactotroph cells of the anterior pituitary, respectively, whereas chorionic somatomammotropin is synthesized in the syncytiotrophoblasts of the placenta (1). Additionally, the availability of clonal lines of rat pituitary tumor cells (GC, GH₃, and GH₄) that produce GH and PRL provides a homologous cell culture system to study the mechanisms that restrict the expression of the GH and PRL genes to the anterior pituitary (2, 3). Thus, the introduction of various lengths of 5' flanking PRL DNA linked to a reporter gene into cultured rat pituitary tumor cells has shown that as few as 250 base pairs (bp) of bovine DNA (2) or as many as 1750 bp of rat DNA (3) are required to impart cell-specific expression to the PRL promoter. Although gene-transfer studies such as these have been crucial in defining cis-acting elements required for tissue-specific gene expression, they do not allow for a detailed biochemical analysis of the transcription factors required.

The development of cell-free systems capable of promoting accurate gene transcription by RNA polymerase II has provided the basis for significant advances in the identification, purification, and characterization of the protein transcription factors (4). Reconstitution of selective gene expression using fractionated cellular extracts has documented the utility of this approach and identified factors required for basal, gene-specific, and enhancer-mediated promoter activity (4). However, few reports have shown that transcriptionally active extracts can be prepared from highly specialized cells to reconstitute cell-specific gene transcription *in vitro*. In this paper we analyze the activity and specificity of the rat PRL (rPRL) promoter in whole cell extracts (WCE) prepared from cultured HeLa and GH₃ rat pituitary tumor cells and demonstrate that pituitary cell-specific factors influence rPRL gene expression *in vitro*. These pituitary factors are able to reconstitute rPRL gene expression in nonpituitary (HeLa) WCE, and we also identified regions of the rPRL promoter that interact selectively with GH₃ pituitary-specific factors by using DNase I protection studies. The ability to recapitulate pituitary-specific gene expression *in vitro* provides a powerful tool to gain insights into the mechanisms that regulate cell-specific gene expression and to assay the biological relevance of DNA–protein interactions.

MATERIALS AND METHODS

Template DNAs. Plasmid DNAs containing either the human α_1 -globin gene (pRB α_1) (5), the Rous sarcoma virus (RSV) promoter linked to the neomycin resistance gene (pRSVneo) (6), or 425 bp of 5' flanking DNA and 5.6 kilobases of transcribed rPRL DNA (exon I through intron C; prPRL-A) (7) were purified by centrifugation in CsCl gradients. DNA concentration was measured by absorbance at 260 nm and was verified by comparison with DNA standards on agarose gels.

Cell-Free Transcription. WCE preparation, cell-free transcription, and RNA purification were performed as described previously (8). The standard 25- μ l reaction contained 15 μ l of HeLa or GH₃ WCE, 1 μ g of uncut plasmid DNA, 1 unit of RNasin (Promega Biotec, Madison, WI) per μ l, each rNTP at 0.6 mM, 8 mM creatine phosphate, 12 mM Hepes (pH 7.9), 60 mM KCl, 7.5 mM MgCl₂, 1.2 mM dithiothreitol, 0.06 mM EDTA, and 10% glycerol and was incubated at 30°C for 120 min. Template DNA was digested with 15 μ g of RNase-free DNase I (Promega) per ml at 37°C for 30 min in 200 μ l of DNase I buffer. DNase I was inactivated by 0.1% NaDodSO₄/5 mM EDTA and several phenol/chloroform extractions. The unlabeled RNA products were then

Abbreviations: PRL, prolactin; rPRL, rat PRL; GH, growth hormone; RSV, Rous sarcoma virus; WCE, whole cell extracts; NE, nuclear extracts.

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coprecipitated with the specific DNA probe (probe structures are shown below Fig. 1 A–C); the ethanol pellets were dried, dissolved in 20 μ l of 0.4 M NaCl/80% formamide/40 mM Pipes, pH 6.4/1 mM EDTA, placed in a boiling water bath for 3 min, and hybridized for 18 hr at the temperatures indicated in Fig. 1. The RNA–DNA hybrids were digested for 30 min at 37°C in S1 buffer (200 μ l) containing 22 units of S1 nuclease (Miles) per μ l, 0.25 M NaCl, 30 mM NaOAc (pH 4.6), and 4.5 mM ZnSO₄. The samples were then phenol/chloroform extracted, ethanol precipitated, dissolved in formamide sample buffer, heated, and electrophoresed on 8% polyacrylamide/8 M urea sequencing gels.

DNase I Protection Assay. A 215-bp DNA fragment spanning the –230 to –16 rPRL region was obtained by *Dde* I and *Pst* I digestion, and the recessed 3' *Dde* I end was filled in with the Klenow fragment of DNA polymerase I and [α -³²P]dGTP in the presence of dATP, dCTP, and dTTP. Each 50- μ l binding reaction contained about 1 ng (35,000 cpm) of end-labeled probe, 350 ng of *Hpa* II-cut pGEM-4 carrier DNA, and the indicated amounts of protein (in μ g) from either WCE or nuclear extracts (NE). The buffer components were the same as the transcription reactions excluding the rNTPs. WCE were prepared as described (8) and NE were prepared as described by Dignam *et al.* (9). Binding was carried out at room temperature for 15 min, and then the samples were diluted with 100 μ l of a solution containing 5 mM CaCl₂ and 10 mM MgCl₂. Each reaction was digested with 20 ng of freshly diluted DNase I (Cooper Biomedical, Malvern, PA) for 60 sec at room temperature. DNase digestion was terminated by the addition of 150 μ l of 0.15 M NaOAc, pH 6.5/1 mM EDTA/0.1% NaDodSO₄/10 μ g of yeast tRNA per ml, and the digested samples were extracted

with phenol/chloroform and then precipitated with ethanol. Labeled DNA pellets were dried, dissolved in formamide sample buffer, and analyzed on 8% sequencing gels that were dried prior to autoradiography.

RESULTS

Cell-Free Transcription in HeLa and GH₃ WCE. As the “neutral” test templates, we chose (i) the human α_1 -globin gene in pRB α_1 (5) because previous studies indicated that this promoter is active both *in vitro* and *in vivo* in heterologous cells (10) and (ii) pRSVneo DNA (6) because the activity of the RSV promoter has been documented in GH₃ pituitary and HeLa cells and because the latter promoter is used often as the neutral promoter in cell-specificity studies (2, 3). Plasmid DNA prPRL-A (7), containing 425 bp of 5' flanking DNA and 5.6 kilobases of transcribed rPRL DNA (exon I through intron C), was used to assess rPRL promoter activity.

Fig. 1A shows that S1 nuclease mapping analysis of the cell-free transcripts directed by pRB α_1 DNA in either HeLa or GH₃ WCE results in a major 159-nucleotide, S1-resistant fragment whose intensity is about equal in HeLa or GH₃ WCE. This fragment maps the *in vitro* initiation site to the authentic *in vivo* cap site (5, 10). Thus, pRB α_1 DNA is transcribed with equal accuracy and efficiency in both extracts. Fig. 1B reveals that the intensity of the 129-bp protected pRSV neo DNA fragment is greater in the GH₃ than in the HeLa cell-free transcription assay. Camper *et al.* (2) have shown by gene transfer studies that the chloramphenicol acetyltransferase activity driven by the RSV promoter appears to be greater in GH₃ cells than in HeLa cells, indicating

FIG. 1. Relative activity and fidelity of various promoters in HeLa and GH₃ WCE. Preparation of WCE, cell-free transcription, and RNA purification were performed as described previously (ref. 8 and *Materials and Methods*). (A) Activity of the human α_1 -globin promoter in HeLa and GH₃ WCE. Transcripts directed by pRB α_1 DNA were quantitated and mapped by using as a probe the 248-bp *Ava* I fragment labeled at the 5' end that contains 159 bp of transcribed DNA (■). Hybridization was performed at 60°C. The 159-nucleotide (nt) protected fragment mapping the α_1 start site is indicated. (B) Activity of the RSV promoter in HeLa and GH₃ WCE. A 5' end-labeled, 1054-bp *Hpa* II fragment that contains 387 bp of pBR322 DNA (---), 538 bp of 5' flanking (—), and 129 bp of transcribed (■) RSVneo DNA was used as probe. The 129-nucleotide protected fragment mapping the authentic pRSVneo cap site is indicated (6, 11). Hybridization temperature was 50°C. (C) Activity of the rPRL promoter in HeLa and GH₃ WCE. A 5' end-labeled, 960-bp *Hpa* II fragment containing 462 bp of pBR322 DNA (---), 425 bp of 5' flanking (—), and 73 bp of transcribed rPRL sequences (■) was used as probe to map rPRL transcripts generated *in vitro*. Hybridization temperature was 52°C. The protected fragment mapping the rPRL RNA start site is indicated. (D) S1 mapping analysis of rPRL transcripts in rat pituitary total RNA. Total RNA was prepared from rat pituitaries as described by White and Bancroft (12). Yeast tRNA was added to each reaction to yield a total of 15 μ g of RNA, and hybridizations with the probe described for C were performed at the indicated temperatures. The lanes labeled “Markers” contain ³²P-labeled, *Hpa* II-cut pBR322 DNA size standards.

that the DNA of the plasmid they used, pRSVcat, is either taken up or transcribed more efficiently in GH₃ cells. Fig. 1B suggests that the transcription efficiency of the RSV promoter is increased in GH₃ cells.

Fig. 1A and B indicate that WCE prepared from cultured rat pituitary tumor cells provide a functional and homologous cell-free transcription system to study rPRL gene transcription. Fig. 1C shows that the cell-free transcription of prPRL-A DNA in HeLa WCE is inactive, whereas in GH₃ pituitary WCE (lane labeled +DNA) it results in three major S1-resistant fragments of about 73, 60, and 52 nucleotides. In the absence of added DNA (lane labeled -DNA), GH₃ WCE do not result in any S1-resistant fragments, indicating that GH₃ WCE do not contain detectable levels of endogenous rPRL mRNA to result in the observed protected fragments. Increasing the prPRL-A DNA concentration from 40 μ g/ml to 120 μ g/ml in HeLa WCE did not reveal any rPRL promoter activity (data not shown). Fig. 1D shows that rat pituitary total RNA yields the same three S1-resistant fragments in a temperature- and RNA-dependent manner, indicating that the cell-free rPRL transcripts are similar to those expressed *in vivo*. The authentic cap site is most likely represented by the 73-nucleotide fragment, since it maps the initiation site about 26 bp downstream from the "TATA" box and has been previously documented by Maurer *et al.* (13). The two smaller fragments may either be due to the S1 assay or they may map alternative initiation sites.

The lack of prPRL-A DNA expression in HeLa WCE suggests either the presence of a rPRL-specific transcription inhibitor, rPRL-specific RNA degradation, the lack of a rPRL-specific stimulatory factor, or a combination of these. To test these possibilities, an extract-mixing experiment was performed as indicated in Fig. 2. Again, specific rPRL gene transcription is absent in HeLa WCE but increases in proportion to the amount of GH₃ WCE added. This result excludes catalytic factors in HeLa WCE that inhibit rPRL gene transcription or decrease rPRL RNA stability; it is consistent either with increasing the concentration of a rPRL-specific stimulatory factor present in GH₃ cells or with diluting a rate-limiting, stoichiometric inhibitory HeLa factor. As little as 3.8 μ l (25%) of GH₃ WCE added to 11.2 μ l (75%) of HeLa WCE reconstitutes rPRL gene transcription. Taken together, the results of Figs. 1 and 2 suggest that cultured GH₃ rat pituitary tumor cells contain cell-specific factor(s) required for selective rPRL gene transcription.

DNase I Footprint Analysis of the rPRL Gene. As a further test for pituitary cell-specific factors that interact with the

rPRL promoter, we compared the DNase I protection pattern of the -230 to -16 rPRL region obtained in GH₃ WCE, GH₃ NE, and HeLa WCE. Since WCE from GH₃ cells exhibit selectivity for the rPRL promoter in the cell-free transcription assay, we initially examined the footprints obtained with increasing amounts of GH₃ WCE and NE (Fig. 3A). Three regions of DNase I protection, labeled footprints I (-45 to -65), II (-120 to -130), and III (beginning at -145), are generated by either GH₃ WCE or NE. Footprint II appears at the lowest concentrations of GH₃ WCE (23 μ g) or NE (9.5 μ g), whereas footprints I and III are dose-dependent. To further assess the cell-type specificity and the rPRL promoter selectivity of the pituitary cell factors, we compared the footprints obtained with GH₃ NE and HeLa WCE and analyzed the effects of unlabeled competitor rPRL DNA (Fig. 3B). HeLa WCE were used because the transcription activity of the rPRL gene has been defined in these extracts; GH₃ NE were used because these extracts result in the same footprints as GH₃ WCE and we also wished to unmask any regions protected by limiting factors in GH₃ WCE. Fig. 3B shows that increasing amounts of GH₃ NE reproduce footprints I and II, define clearly the borders of footprint III (-145 to -175), and reveal a fourth region of weak but evident DNase I protection, footprint IV, that spans nucleotides -190 to about -210. Although footprint IV is abolished by unlabeled rPRL DNA, it may simply be the result of end-binding proteins. Fig. 3A and B also reveal discrete regions of DNase I hypersensitivity that are GH₃-dependent—e.g., surrounding footprint I and on the 3' side of footprint II (near positions -120 to -110). By contrast, a single footprint and an associated hypersensitive region that match the GH₃-induced footprint II domain are observed with HeLa WCE. Footprint II occurs in a dose-dependent manner (135 μ g > 45 μ g) in HeLa WCE (data not shown). Finally, competition with about a 25-fold excess (25 ng) of unlabeled rPRL DNA in GH₃ NE eliminates footprints I, III, and IV and associated DNase I hypersensitive sites but does not eliminate footprint II (lane GH₃ NE).

Sequence Analysis of Pituitary-Specific Footprints of the rPRL Gene. The parallel appearance and disappearance of footprints I, III, and IV in GH₃ extract titration and rPRL DNA competition studies, respectively, suggests that these protected sequences may be homologous and may bind a similar GH₃ cell-specific factor. As shown in Table 1, a sequence comparison of the rat (7, 13), human (14), and bovine (15) PRL genes reveals a conserved sequence motif of 5'-TGAT^{AT}TAA-3' present as a palindrome in footprint I and as a direct repeat in footprint III; these structures are preceded by two highly conserved cytosines. Thus, a single factor is likely to bind the DNA sequences of footprints I and III. Footprint IV contains a less homologous sequence, GCTG-TAATTA, that spans positions -195 to -204. However, since the borders of footprint IV are not well defined and this footprint occurs about 20 bp from the end, its significance is currently difficult to determine.

Footprint II is centered precisely over a 15-bp sequence, GANNTTNNAATTAT, that is highly conserved in the same relative position not only among the various PRL genes (Table 1) but also among all members of the GH gene family, irrespective of species source (1, 13). This region might serve a basal function in transcription of these genes (13). The presence of this factor in nonpituitary (HeLa) cell extracts suggests that it may also bind to other genes.

DISCUSSION

These studies demonstrate that WCE prepared from cultured rat GH₃ pituitary tumor cells selectively and accurately transcribe the rPRL gene and contain cell-specific factors that directly interact with the rPRL promoter. The rPRL gene

FIG. 2. Effects of extract mixing on rPRL gene transcription. The standard cell-free transcription assay was performed as described using prPRL-A template DNA, and the total volume of extract (15 μ l) was held constant. However, the proportion of HeLa to GH₃ extract was varied, as indicated along the top of the gel (% HeLa extract/% GH₃ extract). Specific rPRL transcripts were mapped by S1 analysis, and DNA size markers were as described in Fig. 1.

FIG. 3. (A) DNase I footprint analysis of GH₃ WCE and NE on the rPRL promoter. DNase I footprint analyses were performed with the indicated amounts of added protein (in μ g). Lane M contains ³²P-labeled, *Hpa* II-cut pBR322 DNA size markers. The resultant footprints are labeled I, II, and III. (B) DNase I footprint analysis of HeLa WCE and GH₃ NE on the rPRL promoter. DNase I footprint reactions were performed with the indicated amounts of added protein (in μ g) from either HeLa WCE or GH₃ NE. In this experiment, 100 ng of DNase I was added to the GH₃ reactions and 1000 ng was added to the HeLa reactions in order to obtain a complete digestion ladder. The GH₃ NE lane labeled with an asterisk also contained 25 ng of unlabeled *Dde* I-Pst I rPRL competitor DNA. Lane M contains DNA size markers. The footprints are labeled I-IV, and their relative position on the rPRL 5' flanking DNA probe is depicted as hatched rectangles for the GH₃ cell-specific footprints (I, III, and IV) and as a solid rectangle for the footprint (II) common to HeLa and GH₃ cellular extracts.

is transcriptionally active in GH₃ but not in HeLa WCE (Fig. 1C). The lack of rPRL promoter activity in HeLa WCE is not due to ineffective basal transcription because the HeLa WCE actively transcribe both cellular (human α_1 -globin) and viral (RSV) promoters (Fig. 1A and B). Also, the complementation of rPRL transcription in the HeLa cell-free system by added GH₃ WCE eliminates the possibility of rPRL gene-specific inhibitors in HeLa cells. These data indicate that pituitary cell-specific factors recognize DNA elements contained in the 425 bp of rPRL 5' flanking DNA to reconstitute tissue-specific expression *in vitro*.

Recent gene-transfer studies suggest that as much as 1.7 kilobases of rPRL 5' flanking DNA are required for maximal

gene transcription in cultured rat pituitary cells (3). However, since this DNA region contains an estrogen receptor binding element (16) and the studies reported by Nelson *et al.* (3) were performed in serum-containing medium, the role of tissue-specific versus estrogen-dependent enhancement of rPRL gene expression is not clearly addressed. In contrast, by using clones devoid of the putative estrogen binding region, Camper *et al.* (2) have shown that only 250 bp of 5' flanking bovine PRL DNA is required to impart tissue specificity. The rat and bovine PRL genes display a striking sequence homology in this region (2, 15). Our studies also show that only 425 bp of upstream DNA is sufficient for cell-free, GH₃ selective transcription of the rPRL gene (Fig. 1B).

Table 1. Comparison of rat, human, and bovine PRL sequences in the regions protected from DNase I digestion

Gene	Footprint I		Footprint II		Footprint III	
rPRL	-67	-48	-131	-120	-163	-145
	CCTGATTATATATATTCA		GATGTTTAAAT		CCTGAAATGAATAAGAAA	
hPRL	-63	-46	-129	-118	-160	-143
	CCTGAATC AT TATATTCA		GATGTTTGAAAT		CCTGAATATGAATAAGAA	
bPRL	-62	-45	-128	-117	-160	-141
	CCTGATTA A ATATATTCA		AATGTTTGAAAT		CCTGAATATGAATAAGAAAT	
Consensus	$\xrightarrow{\quad} \text{CCTGA}^{\text{T}}\text{TA(N)} \text{ TATATTCA}$		$\text{G}^{\text{A}}\text{ATGTTTGAAAT}$		$\xrightarrow{\quad} \text{CCTGA}^{\text{T}}\text{ATGAATAAGAA}$	

The rPRL sequences are from Cooke and Baxter (7), the human PRL (hPRL) sequences are from Truong *et al.* (14), the bovine PRL (bPRL) sequences are from Camper *et al.* (15), and the numbering is as indicated by the authors of each report. Spaces are included for maximal alignment. The footprints of the rPRL promoter are listed as footprints I, II, and III, as shown in Fig. 3B. The most highly conserved bases are shown at the bottom as a putative consensus sequence for each footprint, and a comparison of the conserved sequences in footprints I and III result in the common sequence CCTGA^{AT}TA. The orientation of the repeated motif, TGA^{AT}TA, is indicated by the arrows in footprints I and III. The 10-bp sequence of footprint II overlies the 15-bp structure, GANNTTNAAATTAT, previously noted to be conserved among all members of the GH gene family (1, 14).

The current studies have also identified pituitary cell-specific factors present in both GH₃ WCE and NE that selectively bind to the rPRL promoter in a protein-dependent manner. These factors generating footprints I, III, and IV are not present in HeLa cell extracts (Fig. 3B). Recent studies have revealed that they are also absent in Rat-2 fibroblast and C6 rat glioma cells but are present in other sublines of rat pituitary tumor cells (A.G.-H., unpublished results). These pituitary-specific factors bind to the rPRL DNA despite the 350-fold mass excess of nonspecific DNA, suggesting a protein-DNA interaction of high affinity. By contrast, a 25-fold mass excess of specific rPRL competitor DNA completely abolishes binding of factors to the labeled rPRL probe (Fig. 3B), suggesting a highly selective protein-DNA interaction. However, the possibility that two separate regions, footprints I and III, of the rPRL promoter may bind a single factor raises the possibility that they may interact—either competitively or cooperatively.

Whereas these data document rPRL-specific gene transcription and factor binding in GH₃ cell extracts, they do not correlate the requirement of specific factor binding for full activity of the rPRL promoter *in vitro*. Recently, however, Elsholtz *et al.* (17) mapped a cis-acting element that confers transcriptional regulation by both epidermal growth factor and phorbol esters to the -78 to -35 region of the rPRL promoter. Exonuclease footprint analysis revealed a trans-acting factor present in about equal amounts in both unstimulated and hormone-stimulated GH₄ pituitary cells that protected a region of the rPRL promoter overlapping our footprint I (17). One speculation from these data is that epidermal growth factor and phorbol esters mediate augmented rPRL gene transcription by posttranslational modification of a cell-specific factor that binds to the rPRL footprint I region.

The rat GH gene is the only other member of the GH gene family that contains a region homologous to either footprint I or III of the rPRL gene (1). The single rat GH structure is similar to the rPRL footprint III region, overlaps the "CAT" box at positions -88 to -68, and lies within the tissue-specific region (GC-1; positions -94 to -62) recently footprinted by Catanzaro *et al.* (18). This GC-1 region, however, does not significantly affect pituitary-specific rat GH gene expression, but rather it may mediate regulated gene expression (18). By contrast, the rat GH region from -143 to -108 also binds a cell-specific factor (GC-2) and appears to be essential for rat GH gene expression in cultured GC pituitary cells (18). This region coincides with a 15-bp structure that is highly conserved among all members of the GH gene family (1, 14) and is analogous to the rPRL footprint II region (Table 1). Of note, data here show that the factor that binds to this region is present in both HeLa and GH₃ cell extracts and generates a footprint at the lowest concentrations of GH₃ extract used, and this footprint is not eliminated by a 25-fold mass excess of unlabeled rPRL DNA. These results suggest that this factor may be present in significant amounts or may have a very high affinity for rPRL DNA. However, its presence in nonpituitary cell extracts indicates that it may serve a more general role in promoter utilization.

The increased activity of the RSV promoter *in vitro* in GH₃ pituitary versus HeLa cell extracts (Fig. 1B) mimics the *in vivo* observations (2) and further supports the notion that the *in vitro* transcription assay described here recapitulates the tissue-specific expression observed *in vivo*. Interestingly, the RSV promoter region contains the sequence 5'-CCAGATA-

TACGCGTATCTG-3' [spanning positions -349 to -330 (11, 19)], which is about 73% homologous to the footprint I sequence of the rPRL gene. However, the ability of the RSV sequences to bind GH₃-specific factors and to mediate increased transcription in GH₃ cells needs to be tested directly.

Finally, the proximity of footprints I, II, and III suggests that the factors binding to these sequences may interact to result in optimal rPRL transcription. Further studies, using 5' rPRL deletion mutants in the assays reported here, should address this directly and also provide information regarding the similarity of the pituitary cell factors required by the rPRL, rat GH, and RSV promoters. Furthermore, these assays provide a basis for the identification and purification of the pituitary-specific transcription factor(s).

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