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Glucocorticoids are insufficient for neonatal gene induction in the liver

(tyrosine aminotransferase/transcriptional regulation/glucocorticoid receptor/hypoglycemia)

HABIB SASSI, RAYMOND PICTET, AND THIERRY GRANGE*

Institut Jacques Monod du Centre National de la Recherche Scientifique, Université Paris 7, Tour 43, 2 Place Jussieu, 75251 Paris Cedex 05, France

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ABSTRACT **Glucocorticoids and their receptor (GR) play a key role in perinatal gene induction. In the liver, the GR is essential for the neonatal induction of a number of genes, including that coding for tyrosine aminotransferase (TAT). To assess the function of the GR in the perinatal period, we have compared the activity of two types of glucocorticoid responsive elements in transgenic mice; one is the *Tat* gene glucocorticoid-responsive unit (GRU), an assembly of numerous binding sites for transcription factors, including the GR; the other is a simple dimer of high-affinity GR binding sites (GREs). Both elements confer strong glucocorticoid response in the adult liver. However, only the *Tat* GRUs are able to promote neonatal induction; the GRE dimer is unresponsive. Because this dimer is responsive to glucocorticoid administration in the neonate, the absence of neonatal induction is not due to the inactivity of the GR at this stage. At birth, the neonate has to withstand a brief period of starvation and hypoglycemia, a nutritional and hormonal situation that resembles fasting in the adult. In transgenic mice, the responses at birth and after fasting in the adult are similar: the *Tat* GRUs but not the dimeric GREs are activated. Our results show that, in rodents, glucocorticoids are not sufficient for neonatal gene induction in the liver and support the conclusion that the hypoglycemia at birth is the main trigger for expression.**

In adult mammals, glucocorticoid hormones participate in the control of a number of physiological processes, coordinating the response to various stresses, including starvation. During development, glucocorticoids prepare various organs for the major metabolic adaptations that are necessary for autonomous life after birth. Inactivation of the genes coding for the glucocorticoid receptor (GR) and corticotropin-releasing hormone has revealed that, in rodents, the glucocorticoid pathway is essential for survival of the neonate but not of the adult (1, 2). In liver, a number of the genes that are turned on at birth to compensate for the sudden hypoglycemia show an impaired activation profile in the GR knock-out mice (2). This is particularly true for the tyrosine aminotransferase gene (*Tat*), whose transcription is stimulated by both glucocorticoids and glucagon specifically in parenchymal cells of the liver (3).

The GR is a nuclear receptor that can activate or repress transcription of its target genes (for a review, see ref. 4). Transcriptional activation is mediated by the hormone-bound receptor interacting with specific DNA sequences called glucocorticoid-responsive elements (GREs). In natural genes, the GREs are not sufficient for transcriptional activation; the GR must cooperate with other accessory DNA-binding proteins interacting with sites either flanking or overlapping the GREs. This association of GREs with other transcription factor binding sites has been called a glucocorticoid-responsive unit (GRU; ref. 5) or a composite GRE (6, 7). This association makes it possible to integrate the glucocorticoid response with other regulatory pathways (6–10). The GRUs of the *Tat* gene show such characteristics. The gene is activated through cooperative interaction of two GRUs located at −2.5 kb and −5.5 kb. These GRUs consist of numerous contiguous and overlapping binding sites for the GR and transcription factors of the C/EBP, HNF-3, and Ets families (9, 11, 12). This arrangement confers tissue specificity to the glucocorticoid response and allows positive synergism between the glucocorticoid and glucagon pathways and negative synergism with the insulin pathway (10, 13, 14).

The requirement for cooperation between the GR and other transcription factors can be alleviated, at least in part, by combining two high-affinity GR binding sites, optimally spaced with two helical turns between the center of each site (15). Such dimeric GREs should reveal the transcriptional activation potency of the GR because it does not depend on other accessory DNA-binding proteins. The limit to this expectation is that the dimeric GREs require both a minimal promoter for transcription initiation and, when tested as integrated copies in transgenic animals, an enhancer to turn on the gene above a certain threshold (ref. 16, see also ref. 14). This arrangement creates a novel combination of regulatory elements that in effect may reconstruct a GRU. To study the function of the GR in various tissues and developmental stages, the contribution of other regulatory pathways should be minimized as much as possible. In theory, this could be achieved by using a promoter and an enhancer originating from an ubiquitously expressed gene. We have used such an experimental strategy to analyze the activity of the GRUs of the *Tat* gene in transgenic mice (14). These GRUs were placed upstream of the promoter of the gene coding for the largest subunit of RNA polymerase II *(RpII)*. By using transient expression assays in cultured cells, we showed that the GRUs regulate optimally the *RpII* promoter, while preserving its full basal activity, when inserted between the minimal promoter and the enhancer (14). In transgenic mice, a reporter gene under the control of these regulatory elements showed the ubiquitous expression pattern characteristic of the *RpII* gene and the tissue-specific glucocorticoid response provided by the *Tat* GRUs (14). To analyze the transcriptional activation potency of the GR *in vivo*, we have now inserted dimeric GREs within the *RpII* promoter and created transgenic mice with this construct. We have used these transgenic mice to study the function of the GR in liver in the perinatal period. The comparison of the responses mediated by the *Tat* GRUs and

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Abbreviations: GR, glucocorticoid receptor; GRE, glucocorticoid-responsive element; GRU, glucocorticoid-responsive unit; TAT, tyrosine aminotransferase; CAT, chloramphenicol acetyltransferase; RPII, largest subunit of RNA polymerase II; AFP, α-fetoprotein; PKA, protein kinase A.

*To whom reprint requests should be addressed. e-mail: grange@ccr.jussieu.fr.

the dimeric GREs allows us to conclude that glucocorticoids are not sufficient for neonatal gene induction in the liver and indicates that the hypoglycemia at birth is the main trigger.

MATERIALS AND METHODS

DNAs Used for Transgenesis. Standard recombinant DNA procedures were used (17). The construct containing the two GRUs of the *Tat* gene inserted in the *RpII* promoter was as described (14). It contains the chloramphenicol acetyltransferase gene (*Cat*) as a reporter gene and splicing and polyadenylylation sites originating from the rabbit β-globin gene. The construct containing the GRE dimer was derived from this as follows: a *Hin*dIII fragment containing the two *Tat* gene GRUs, the proximal *RpII* promoter (positions −51 to +93) and the *Cat* reporter gene was replaced by a *Bsp*1286I–*Bgl*I fragment originating from p2GM (11) and containing the GRE dimer, the proximal *RpII* promoter (positions −51 to +93), and the *Cat* reporter gene.

Production and Analysis of Transgenic Mice. The DNA fragment for microinjection was separated from the vector sequence and purified on an agarose gel followed by passage over an Elutip column (Schleicher & Schuell). About 400 copies of the purified fragment were microinjected into the male pronuclei of fertilized C57BL/6J × DBA/2J eggs and the microinjected eggs were subsequently transferred to the oviduct of pseudopregnant mice by standard procedures (18). Transgenic mice were identified by slot-blot analysis of tail DNA. To define the structure of each integrated transgene, Southern blots were carried out with *Pvu*II-digested genomic DNA and a *Cat* probe. Transgenic lines were established in the C57BL/6J × DBA/2J background.

The progenies of transgenic founders were used for expression analyses. Mice were trained with a regular dark/light cycle (dark phase, hours 1800–0600). Transgenic littermates (adults, 6 weeks old) were separated in two groups: at 0900, mice of the first group were injected intraperitoneally with 2 μg of dexamethasone per g of body weight, whereas mice of the second group were injected with a 0.9% NaCl solution as a control. The livers were collected 6 h later. In addition, another group of mice was analyzed independently after fasting for 48 h (the food was removed at 1400). For perinatal transgene expression analyses, transgenic males from each line of GRUs or GRE mice were mated with wild-type females. Successfully mated females were scored the day after their transfer to the male cage. Plug detection was designated day 1 of gestation. On embryonic day 19, one group of females was sacrificed, and fetuses were collected. The newborns of the other group were sacrificed 6 h after birth. To analyze the glucocorticoid response in the postnatal period, mice were processed as for the analysis in the adult. The weaning of the mice analyzed 21 days after birth was initiated by separation from their mother on day 18.

The extracts were prepared and analyzed as follows. The livers were washed in 1× PBS and homogenized in 1 ml of 250 mM Tris·HCl, pH 7.5/5 mM EDTA/5 mM DTT, either directly or after storage in liquid nitrogen. The homogenate was centrifuged for 10 min at 4°C in an Eppendorf centrifuge, and the supernatant was heated at 65°C for 10 min and centrifuged again for 10 min at 4°C. CAT activities and total protein were measured by standard procedures (17).

RESULTS

A Dimer of a GRE Confers a Strong Glucocorticoid Response in the Adult Liver. The major high-affinity GR binding site in the −2.5 kb GRU of the *Tat* gene is a typical palindrome with a 3-bp spacing (19). In transient transfection assays, it can confer glucocorticoid response to a linked promoter when duplicated (15). The strongest activation is achieved when sites are spaced with a center to center distance of 21 bp that is optimal for synergistic binding of GR to DNA (15). This high-affinity GR-binding site also binds members of the Ets family of transcription factors, and these factors contribute to the transcriptional activation mediated by this dimerized site (11). A single base mutation in the 3-bp spacer of the palindrome can selectively abrogate Ets binding without affecting the glucocorticoid response (11). We have inserted such a mutated dimer, hereafter referred to as $(GRE)_2$, into the *RpII* promoter at position −52. This position has been shown to be optimal to observe the glucocorticoid induction of the promoter by the *Tat* GRUs (14). The chimeric promoter drives the expression of the *Cat* reporter gene (Fig. 1).

Four transgenic mouse lines were generated with this $(GRE)_2$ construct and the presence of transgenic DNA was analyzed along with transgene expression (Table 1). Two of these lines expressed the transgene in the tail, an expression pattern that proved to be diagnostic of the activity of the *RpII* promoter (14). Our previous analysis of the *RpII* promoter controlled by the *Tat* GRUs had shown that out of five transgenic lines, three expressed the transgene in all tissues, including the tail, whereas the two others showed no expression in any tissue (ref. 14 and Table 1). Thus, the proportion of expressing lines is similar with the two constructs.

We have analyzed transgene expression in two lines obtained with the $(GRE)_2$ and two lines obtained with the *Tat* GRUs similarly embedded in the *RpII* promoter (Fig. 1). Both the artificial $(GRE)_2$ and the natural GRUs confer a strong glucocorticoid response in liver (Table 2). The GRUs conferred a 35-fold induction in both lines. The response is even higher for the $(GRE)_2$, which provides a fold induction by dexamethasone ranging from 50 (line 35) to 120 (line 5). In conclusion, the simple artificial GRE dimer with an optimal spacing is a very efficient glucocorticoid responsive modulator of transcription *in vivo*.

The *Tat* GRUs but Not the $(GRE)_2$ Confer Gene Induction at Birth in the Liver. To study the role of glucocorticoids on neonatal gene induction, we measured the CAT activity in the liver of transgenic mice at embryonic day 19, i.e., 1 day before birth, and also 6 h after parturition. Fig. 2 shows that none of the $(GRE)_2$ lines have increased CAT activity after birth, whereas both GRU lines show a strong perinatal induction, about 20-fold for line 29 and about 50-fold for line 33. Thus, the GRE dimer that is a very efficient glucocorticoid respon-

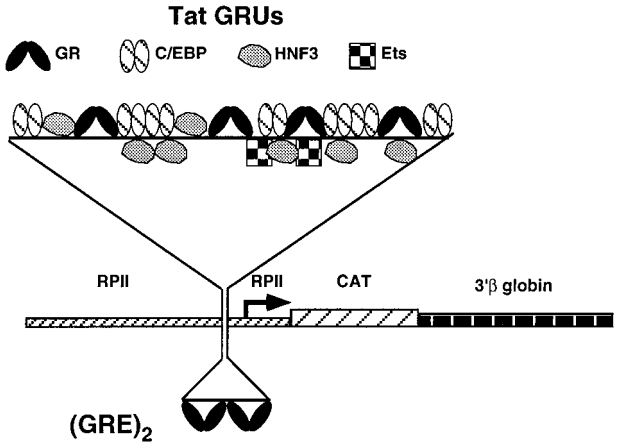

FIG. 1. Schematic representation of the two constructs used for transgenesis. The arrangement of the transcription factor binding sites in the glucocorticoid regulated enhancers is shown by the presence of the corresponding factors, represented by symbols as indicated. The three differently hatched boxes represent the other segments shared by the two constructs; RPII, RNA polymerase II gene promoter; CAT, CAT coding region; 3′ β-globin, last intron and polyadenylylation site of the rabbit β-globin gene (for details, see ref. 14).

Table 1. Transgene integration and expression in tails from transgenic mice of independent lines

Sequence	Line	Copy number	CAT activity
$(GRE)_2$	5	2	220 ± 8 (13)
	6	1	No expression
	7	2	No expression
	35	2	127 ± 10 (10)
GRUs	28	2	No expression
	29	2	211 ± 35 (5)
	33	5	389 ± 50 (4)
	40	1	No expression
	41	2	103 ± 23 (3)

Copy number was estimated by both slot-blot and Southern blot analyses of mouse tail DNA. The CAT activity (pmol of chloramphenicol acetylated per min per μg of protein) (mean ± SEM) measured in the tail is indicated; number of mice analyzed is in parentheses.

sive modulator in the adult liver is unresponsive to the modification of the hormonal balance that takes place at birth in mice. In contrast, the *Tat* GRUs integrate the glucocorticoid response to other regulatory stimuli that confer the neonatal gene induction that is characteristic of the *Tat* gene (20).

The *Tat* GRUs but Not the $(GRE)_2$ Confer the Response to Starvation in the Liver. Immediately after birth, the newborn has to withstand a brief period of starvation that is accompanied by an increase in plasma glucagon and a decrease in plasma insulin (21). *Tat* gene transcription is stimulated by glucagon, acting through protein kinase A (PKA) stimulation (3). In cultured cells, PKA acts through at least two elements: a cAMP response unit at position −3.6 kbp and the GRU at position −2.5 kbp, and the PKA effect on both units is counteracted by insulin (10, 13, 22). This suggests that the *Tat* GRUs could mediate *in vivo* a response to an inversion of the insulin/glucagon ratio, thus accounting for the neonatal gene induction observed. To test this possibility, transgenic adults were subjected to a 48-h fasting period to mimic the change of the insulin/glucagon ratio occurring at birth. CAT activities measured in the liver of the fasted animals were compared with the activities measured in the perinatal period or after dexamethasone administration to the adult (Fig. 3). The results show that the GRUs are even more responsive to starvation than to dexamethasone injection. In contrast, the GRE dimer is essentially unresponsive to fasting. In conclusion, the pattern of neonatal gene induction parallels the response to hypoglycemia but does not parallel that to glucocorticoids alone.

The $(GRE)_2$ Is Responsive to Glucocorticoids in the Postnatal Period. The absence of neonatal gene induction conferred by the $(GRE)_2$ could have been due to the inability of the GR to activate, at this developmental stage, the basal transcriptional machinery interacting with the *RpII* promoter. To test the functionality of all components of the response pathway, we administered dexamethasone to pups of transgenic line 5 at birth and 1 or 21 days after birth (i.e., 3 days after weaning). The results show that the $(GRE)_2$ is responsive to dexamethasone in the perinatal period, even though the induced level is not as strong as it is in the adult (6 weeks old; Fig. 4). This induced level does not vary in early life and is not enhanced 3 days after weaning, showing that the metabolic adaptations occurring at this stage are not responsible for the increased glucocorticoid responsiveness observed in the adult. The basal level is slightly higher in the perinatal period and then declines in the days after birth to reach the adult level. This pattern of expression is presumably due to endogenous glucocorticoids that peak 2 days before birth and decline thereafter (for review, see refs. 21 and 23).

Table 2. Fold induction of CAT activity by dexamethasone in the adult liver of four transgenic lines

Sequence	Line	R. I. Dex
$(GRE)_2$	5	116 ± 15 (4)
	35	53 ± 15 (3)
GRUs	29	36 ± 8 (3)
	33	35 ± 6 (4)

The fold induction of CAT activity by dexamethasone (as a ratio of induced to uninduced level, R. I. Dex; mean ± SEM) is indicated. The number of pairs of mice analyzed is in parentheses.

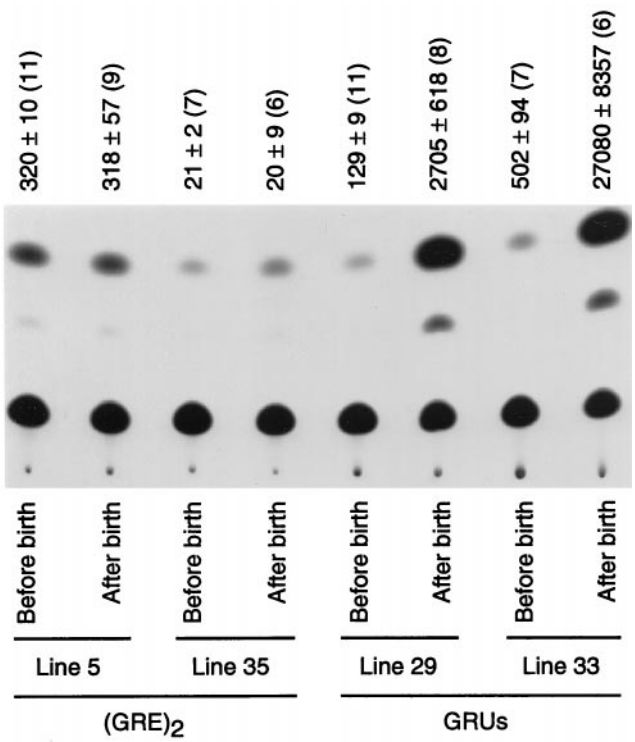

FIG. 2. In the liver, the *Tat* GRUs but not the $(GRE)_2$ confer gene induction at birth. CAT assays for a representative pair of mice from two $(GRE)_2$ lines (lines 5 and 35) and two GRUs lines (lines 29 and 33) are shown. Before birth, embryonic day 19; after birth, 6 h after parturition. The amount of liver extract protein used was 10 μg (line 33), 40 μg (lines 5 and 29), or 100 μg (line 35). The reactions were incubated at 37°C for either 10 min (lines 5, 29, and 33) or 20 min (line 35). Above each assay, the CAT activity (mean ± SEM) measured is indicated, as well as the number of mice analyzed in parenthesis.

DISCUSSION

In the early 1970s, Greengard (20, 24) noted that, in the final stages of liver development, a number of enzymes are induced simultaneously. These enzymes have been grouped in three clusters according to their timing of induction: the late fetal cluster, the neonatal cluster, and the late suckling cluster. Greengard argued that certain hormones were the triggers of these inductions because their level showed the appropriate fluctuation at the time of induction and because their administration could evoke premature induction (20, 24). On the basis of these criteria, she proposed that glucocorticoids are an important trigger for the induction of the late fetal and suckling clusters, whereas glucagon is the main trigger for induction of the neonatal cluster (20, 23, 24). In agreement with this proposition, the *Tat* gene, which belongs to the neonatal cluster, can be prematurely induced by glucagon in the 3 days that precede birth (25). In contrast, it was observed in 1959 that glucocorticoids are not able to elicit premature prenatal *Tat* gene induction even though they play an important permissive role in neonatal induction (26). This impor-

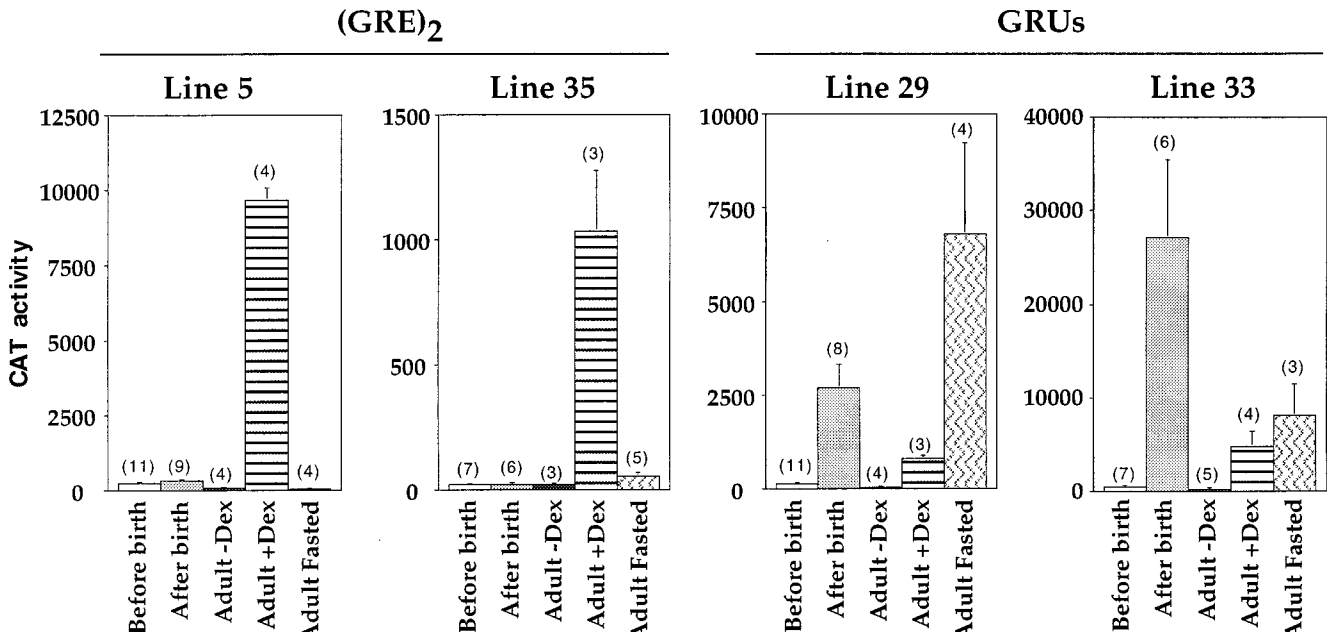

FIG. 3. In the liver, gene induction at birth and response to hypoglycemia is conferred by the *Tat* GRUs but not by the $(GRE)_2$. The CAT activity (mean ± SEM) is represented as bars. The number of individuals tested is indicated between brackets.

tance of glucocorticoids has been confirmed with the modern tools of molecular genetics by Schütz and collaborators (2): neonatal *Tat* gene induction is impaired in GR knock-out mice.

We show herein that glucocorticoids are not able to induce neonatal gene expression in rodents. Our conclusion relies on the response mediated *in vivo* by a dimeric GRE inserted between the minimal promoter and enhancer originating from the ubiquitously expressed *RpII* gene. The GRE dimer does not provide neonatal gene induction even though it responds very strongly to glucocorticoid injection (up to 120-fold induction). In contrast, the *Tat* GRUs inserted into the same environment as the dimeric GRE are able to confer neonatal gene induction, showing that the *RpII* promoter and enhancer are able to sustain this activation.

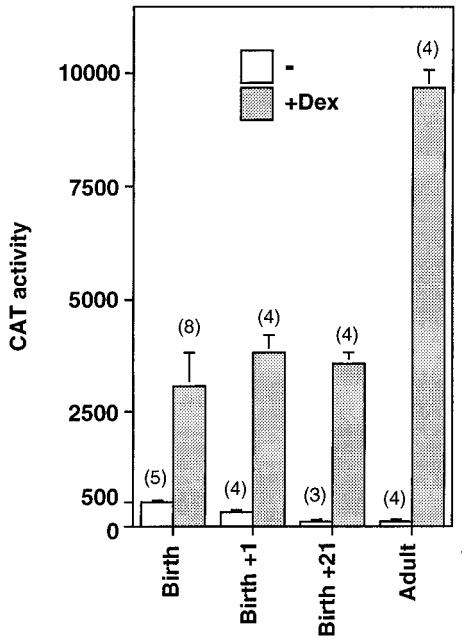

FIG. 4. The $(GRE)_2$ is responsive to glucocorticoids in the postnatal period. The results correspond to the analysis of livers from the $(GRE)_2$ transgenic line 5 and are presented as indicated in Fig. 3. Animals were either injected with saline (−Dex) or dexamethasone (+Dex) and analyzed 6 h later. Birth +1 and Birth +21 are animals injected, respectively, 24 h or 21 days after birth.

As proposed by Greengard (20, 24), the hypoglycemia at birth is likely to be the trigger for developmental induction of the neonatal cluster in liver since glucose injection prevents this increase. The *Tat* GRUs appear to contain the information necessary to respond to this stimulus as they confer both neonatal induction and response to fasting in the adult. It is likely that the inversion of the insulin/glucagon ratio in these situations plays a key role in the response. Indeed, in cultured cells, the *Tat* GRUs allow the integration of the glucocorticoid response to these hormonal pathways: this complex element confers positive synergism with PKA (the downstream target of glucagon) and negative synergism with insulin (10, 13). The *Tat* GRUs consist of numerous contiguous and overlapping binding sites for the GR and transcription factors of the C/EBP, HNF-3, and Ets families (9, 11, 12). C/EBP and HNF-3 are good candidates for mediating the response to hypoglycemia. C/EBP isoforms have been implicated in transcriptional activation by cAMP (27, 28), and HNF-3 participates both in the cAMP stimulation of the glucocorticoid response conferred by the *Tat* GRUs and in the negative effect of insulin (10, 13). A similar response is also conferred by the GRU of the carbamyl phosphate synthetase gene that contains binding sites for GR, C/EBP, and HNF-3, further emphasizing the importance of these factors (V. Christoffels, T.G., and W. Lamers, unpublished results). In contrast, CREB, a well-known mediator of the cAMP response, does not appear to be involved because it does not interact with the *Tat* GRUs (ref. 9 and data not shown). In the *Tat* gene, a CREB binding site located in a cAMP responsive unit at −3.6 kb has been shown to be involved in the response to cAMP (22). However, as indicated herein, this unit is dispensable for the developmental and hormonal pattern of expression of the gene.

Because glucocorticoids are important for neonatal *Tat* gene induction (2, 26), what role does the GR play in the activation of the *Tat* GRUs? In cultured cells, glucocorticoids play a dominant role in the regulatory hierarchy: They are essential to the activity of the *Tat* GRU at position −2.5 kb presumably because they trigger a local chromatin remodeling, as well as the recruitment of HNF-3 and C/EBP (refs. 9, 10,

and 29 and H. Thomassin and T.G., unpublished results). Such a dominant role could explain why these hormones are necessary, although not sufficient, for neonatal *Tat* gene induction. The glucocorticoid level peaks 2–3 days before birth and is in a descending phase at birth (30, 31). This peak could be sufficient to allow the action of the other transcription factors that induce the gene at birth. The peak also could be responsible for the slightly enhanced activity of the dimeric GRE that we observed throughout the perinatal period.

Our observations using the dimeric GRE differ from the recent transgenic analysis of Montoliu *et al.* (16) that showed neonatal induction (20- to 30-fold) of a reporter gene driven by a dimeric GRE inserted between a minimal herpes simplex virus thymidine kinase promoter and enhancers originating from the liver-specific α-fetoprotein (*Afp*) gene. Such a neonatal induction was surprising because this reporter is poorly induced by glucocorticoid injection (6-fold induction) in contrast to our GRE-*RpII* construct (up to 120-fold), and because in rodents glucocorticoids peak 2–3 days before birth and are in a descending phase at birth (23, 30, 31). The discrepancy between the two transgenic analyses is likely to be due to the action of factors binding to sequences neighboring the GRE. Interestingly, the same set of transcription factors interacts with the two regulatory sequences that confer neonatal induction, the *Tat* GRUs and the sequences used in the transgenic study of Montoliu *et al.* (16): the dimeric GRE they used is also a dimeric Ets-binding site (11) and the *Afp* enhancers contain numerous binding sites for C/EBP and HNF-3 that could synergize with this dimeric GRE (32–34). Such an interpretation is supported by the observation that the *Afp* sequences contribute to the expression patterns of the construct in the liver, because a typical predominance in the pericentral region was seen (16). Therefore, these transcription factors, and not the GR by itself, are likely to be responsible for the perinatal induction of a GRU fortuitously reconstituted in the transgene of Montoliu *et al.* (16).

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1. Muglia, L., Jacobson, L., Dikkes, P. & Majzoub, J. A. (1995) *Nature (London)* **373,** 427–432.
2. Cole, T. J., Blendy, J. A., Monaghan, A. P., Krieglstein, K., Schmid, W., Aguzzi, A., Fantuzzi, G., Hummler, E., Unsicker, K. & Schütz, G. (1995) *Genes Dev.* **9,** 1608–1621.
3. Granner, D. K. & Hargrove, J. L. (1983) *Mol. Cell. Biochem.* **53/54,** 113–128.
4. Beato, M., Herrlich, P. & Schütz, G. (1995) *Cell* **83,** 851–857.
5. Schüle, R., Muller, M., Kaltschmid, C. & Renkawitz, R. (1988) *Science* **242,** 1418–1420.
6. Diamond, M. I., Miner, J. N., Yoshinaga, S. K. & Yamamoto, K. R. (1990) *Science* **249,** 1266–1272.
7. Miner, J. N. & Yamamoto, K. R. (1991) *Trends Biochem. Sci.* **16,** 423–426.
8. Imai, E., Stromstedt, P., Quinn, P., Carlstedtduke, J., Gustafsson, J. & Granner, D. (1990) *Mol. Cell. Biol.* **10,** 4712–4719.
9. Grange, T., Roux, J., Rigaud, G. & Pictet, R. (1991) *Nucleic Acids Res.* **19,** 131–139.
10. Espinás, M. L., Roux, J., Pictet, R. & Grange, T. (1995) *Mol. Cell. Biol.* **15,** 5346–5354.
11. Espinás, M., Roux, J., Ghysdael, J., Pictet, R. & Grange, T. (1994) *Mol. Cell. Biol.* **14,** 4116–4125.
12. Roux, J., Pictet, R. & Grange, T. (1995) *DNA Cell. Biol.* **14,** 385–396.
13. Ganss, R., Weih, F. & Schütz, G. (1994) *Mol. Endocrinol.* **8,** 895–903.
14. Sassi, H., Fromont, R. M., Grange, T. & Pictet, R. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 7197–7201.
15. Schmid, W., Strahle, U., Schütz, G., Schmitt, J. & Stunnenberg, H. (1989) *EMBO J.* **8,** 2257–2263.
16. Montoliu, L., Blendy, J. A., Cole, T. J. & Schütz, G. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 4244–4248.
17. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.
18. Hogan, B., Costantini, F. & Lacy, E. (1986) *Manipulating the Mouse Embryo: A Laboratory Manuel* (Cold Spring Harbor Lab. Press, Plainview, NY).
19. Jantzen, H. M., Strähle, U., Gloss, B., Stewart, F., Schmid, W., Boshart, M., Miksicek, R. & Schütz, G. (1987) *Cell* **49,** 29–38.
20. Greengard, O. (1970) in *Biochemical Actions of Hormones*, ed. Litwack, G. (Academic, New York), Vol. 1, pp. 53–87.
21. Girard, J., Ferré, P., Pégorier, J. P. & Duée, P. H. (1992) *Physiol. Rev.* **72,** 507–562.
22. Boshart, M., Weih, F., Schmidt, A., Fournier, R. E. & Schütz, G. (1990) *Cell* **61,** 905–916.
23. Greengard, O. (1975) *J. Steroid Biochem.* **6,** 639–642.
24. Greengard, O. (1971) *Essays Biochem.* **7,** 159–205.
25. Greengard, O. & Dewey, H. K. (1967) *J. Biol. Chem.* **242,** 2986–2991.
26. Sereni, F., Kenney, F. T. & Kretchmer, N. T. (1959) *J. Biol. Chem.* **234,** 609–612.
27. Metz, R. & Ziff, E. (1991) *Genes Dev.* **5,** 1754–1766.
28. Roesler, W. J., Crosson, S. M., Vinson, C. & McFie, P. J. (1996) *J. Biol. Chem.* **271,** 8068–8074.
29. Rigaud, G., Roux, J., Pictet, R. & Grange, T. (1991) *Cell* **67,** 977–986.
30. Henning, S. J. (1978) *Am. J. Physiol.* **235,** 451–456.
31. Dalle, M., Giry, J., Gay, M. & Delost, P. (1978) *J. Endocrinol.* **76,** 303–309.
32. Godbout, R., Ingram, R. & Tilghman, S. M. (1988) *Mol. Cell. Biol.* **8,** 1169–1178.
33. Millonig, J. H., Emerson, J. A., Levorse, J. M. & Tilghman, S. M. (1995) *Mol. Cell. Biol.* **15,** 3848–3856.
34. Thomassin, H., Bois-Joyeux, B., Delille, R., Ikonomova, R. & Danan, J. L. (1996) *DNA Cell. Biol.* **15,** 1063–1074.