Glucocorticoid Inhibition of Gene Expression and Proliferation of Murine Lymphoid Cells in Vitro

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Abstract

Glucocorticoids inhibit the proliferation of murine T-lymphoma P1798 cells. P1798 cells do not die in the presence of dexamethasone, and the process of inhibition of proliferation is completely reversible. As cells cease to divide, expression of a number of genes is inhibited. Among these are genes the expression of which is regulated in some manner that is linked to cell proliferation. We have undertaken to study the mechanism whereby glucocorticoids inhibit the expression of genes in P1798 cells. Three model systems will be reviewed. In all cases, these appear to be examples of secondary regulation. Glucocorticoid-mediated inhibition of transcription of the DNA encoding ribosomal RNA (rDNA) has been investigated in some detail. The data indicate that dexamethasone causes a decrease in the amount or activity of an RNA polymerase I transcription initiation factor. This factor exhibits a short biological half-life and the data are consistent with the hypothesis that glucocorticoids regulate the synthesis of this transcription factor. The gene encoding thymidine kinase appears to be regulated in a similar fashion. On this basis, we propose that glucocorticoids may have the general property of regulating the synthesis of certain transcription factors. Glucocorticoids also regulate the translation of a certain class of mRNAs, including those that encode ribosomal proteins. These are characterized by a low efficiency of translation in untreated cells. Upon exposure to dexamethasone, the translation of these mRNAs is disproportionately inhibited. We speculate that translation of the mRNAs encoding certain transcription factors may be regulated in a similar fashion. Specifically, we propose that transcription of certain proliferation-related genes may be dependent upon factors of short biological half-life. These are encoded by mRNAs that are poorly translated under optimal growth conditions. Any slight perturbation in translation efficiency, as caused by glucocorticoids, results in a disproportionate inhibition of synthesis of these hypothetical transcription factors. Transcription of a class of proliferation-related genes ceases as a result.

Introduction

The prevailing hypothesis concerning the mechanism of action of glucocorticoids (and other steroid hormones) states that all of the effects of these agonists are mediated by a high affinity receptor and are attributable to changes in the pattern of gene expression in target cells. This hypothesis is supported by a body of genetic and biochemical data, and the glucocorticoid receptor is among the best characterized mammalian transcription factors. A great deal is known concerning the structure and properties of this entity. Less is known about the way in which the receptor interacts with DNA and the transcriptional machinery. However, the state of the technology is such that it is reasonable to expect reconstitution in vitro of hormonal induction of one or another target gene. From such studies will emerge a more detailed understanding of the biochemical mechanism(s) that underlie stimulation of transcription by steroid hormones.

The salient question for the future resolves itself in terms of the physiological consequences of the interaction between the steroid hormone receptor and its genomic target sites. Our laboratory is committed to one aspect of this question: the relationship between hormonal regulation of gene expression and cell proliferation. Specifically, we have undertaken to explore those mechanisms that account for inhibition of proliferation of lymphoid cells by the synthetic glucocorticoid dexamethasone. The experimental system that has been used is the murine T-lymphoma line P1798. As described below, these cells are subject to reversible, glucocorticoid receptor-mediated inhibition of proliferation.

As P1798 cells cease to divide in the presence of dexamethasone, one observes a striking change in the pattern of gene expression. The most notable effect is inhibition of expression of a large number of genes. Among these are genes the products of which are thought to be essential for progression through the cell cycle. Causality remains to be determined. It cannot be said with certainty whether inhibition of expression of any particular gene causes or results from inhibition of proliferation. Nevertheless, the observation that a battery of proliferation-related genes is turned off suggests considerable physiological significance. Furthermore, the net effect of glucocorticoids upon these cells appears to be inhibitory, and little is known about the mechanisms whereby gene expression is inhibited by steroids.

To address this phenomenon, the following working hypothesis has been proposed. There are certain key metabolic processes that determine the rate at which cells divide. In some cell types, these processes are regulated by glucocorticoids (as well as other mitotic stimuli). The activity of these hypothetical processes is directly related to the rate of transcription of a large group of genes. Stating this less generally, we propose that glucocorticoids directly regulate the expression of a small number of genes. The products of these genes regulate, either directly or indirectly, the expression of a larger number of secondary loci. The products of the primary interactions serve, therefore, to amplify the response and to coordinate subsequent regulatory transitions such as might be expected to attend a major alteration in cellular physiology (e.g., a reversible change in the rate of proliferation).

To test this hypothesis, our immediate aims have been to identify the most distal loci and to investigate the biochemical details of the mechanisms that account for their regulation by glucocorticoids. This experimental approach is perceived as a first step in unraveling the regulatory cascade. The long term objective is to elucidate the pathway that leads from the primary interaction to the ultimate physiological consequences of exposure to glucocorticoids.

In this paper, we have undertaken to review some of the data that have contributed to our view of hormonal inhibition of cell proliferation. The phenomenon of secondary inhibition of transcription will be presented along with recent data concerning posttranscriptional regulation of gene expression by glucocorticoids. We will speculate concerning the possible relationship between these two phenomena and address the tentative hypothesis that translational regulation by glucocorticoids may account for coordinate inhibition of transcription of a large number of genes.
Materials and Methods

P1798 cell lines were established in culture from the transplantable tumor line identified as lymphosarcoma P1798 (1). Cells were cloned by limiting dilution and are routinely maintained in suspension culture at 37°C in RPMI 1640 supplemented with 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 20 mM β-mercaptoethanol, and 5% fetal bovine serum. All cell culture reagents were purchased from commercial sources.

Measurement of mRNA abundance was carried out using unfraccionated cellular RNA isolated using sodium dodecyl sulfate and proteinase K (2). Electrophoretic resolution of denatured RNA, blotting, and hybridization were carried out as described previously (3). Autoradiographic exposure was normalized to ensure that all signals fell within the linear range of exposure, and analysis of autoradiographic signal intensity was carried out using a Beckman DU8 spectrophotometer (4). Enzymatic activity was determined using published procedures (5). Protein concentration was measured by the method described by Bradford (6).

Transcription in vitro was carried out using the cloned mouse promoter-containing plasmid designated prMAB (7). Preparation of S100 extracts and reaction conditions are described elsewhere (7). Measurement of stable, preinitiation complex formation was carried out by order of addition, template exclusion protocols (5). Formation of heparin-resistant transcription complexes was assayed by a modification of a published procedure (8). The nature of this modification will be described elsewhere. The purification and properties of the rDNA transcription factor TFIC will be described in a subsequent publication.

Polysomal distribution of ribosomal protein mRNAs was measured by Dr. Oded Meyuhas in collaboration with Dr. Robert Perry. Isolation and resolution of ribosomes and quantitation of mRNAs are described in a separate publication (9).

Results

Growth of P1798 Cells in Culture. Representative growth curves are shown in Fig. 1. It is characteristic of P1798 cells that they exhibit a lag upon subculturing. The population-doubling time during the first day after subculturing is ~24 h. Most of the experiments described below were done under these conditions. After 24 h in culture, the population-doubling times decrease to the extent that log phase cultures double with a mean time of 9–11 h. Under certain conditions, doubling times of 4–6 h can be achieved.

Glucocorticoids inhibit proliferation of wild-type P1798 cells in culture. Addition of 0.1 μM dexamethasone at the time of subculturing (0 h in Fig. 1) precludes any subsequent cell division. If dexamethasone is added to log phase cultures (24 h in Fig. 1), one net doubling is observed within the ensuing 24 h. The culture densities remain constant thereafter.

Dexamethasone does not kill P1798 cells. As illustrated in Fig. 2, the apparent viability (as assessed by dye exclusion) remains high (>95%) for 96 h in 0.1 μM dexamethasone. Wehi 7 cells, which exhibit the classical lytic phenotype, die under conditions identical to those which do not kill P1798. The lytic phenotype is expressed by P1798 cells as an epigenetic phenomenon, and glucocorticoid-mediated cell death can be observed in vivo and under certain conditions in culture (10). All of the experiments described below were done under conditions that provided for >97% viability for the duration of the experimental protocol.

Glucocorticoid inhibition of P1798 proliferation is a reversible phenomenon (1, 11). Treatment for 24 h does not significantly impair the ability of these cells to reinitiate DNA synthesis within 24 h after removal of dexamethasone. However, prolonged treatment causes a significant reduction in the rate at which cells enter S phase after removal of the steroid. The significance of this observation is unclear. This may be due to permanent arrest (i.e., differentiation) or to delayed reentry into the cell cycle. Nevertheless, it is clear that cultures can be rescued after treatment for 5 days. Such rescued cultures retain the sensitive phenotype (1). The experiments described below were carried out under conditions that provided for complete reversibility of growth arrest.

Glucocorticoid Effects upon Gene Expression. Glucocorticoids cause reversible G1 arrest of wild-type P1798 cells (3). This is associated with profound changes in the metabolic activity of the cells. Corresponding changes are observed in the pattern of expression of certain proliferation-related genes. Some of these data are summarized in Fig. 3. The most rapid response detected to date is inhibition of expression of c-myc. The abundance of c-myc mRNA decreases rapidly with 50% inhibition observed within 90 min. This is associated with decreased transcription of the protooncogene (3). From a quantitative standpoint, the most significant effect is inhibition of synthesis of rRNA. Fifty % inhibition is observed within ~6 h and >95% inhibition occurs within 24 h. In mid-log phase cells, this results in 60–80% inhibition of incorporation of [3H]uridine and >50% inhibition of total nuclear transcriptional activity.

Expression of thymidine kinase (TK) is also inhibited. This response occurs relatively slowly (t1/2 ≈ 12 h). The abundance of other mRNAs (notably that encoding thymidylate synthase) decreases somewhat later (5). Expression of a large number of genes is unaffected. These include actin (Fig. 3), glucose phosphate isomerase (11), superoxide dismutase (9), hypoxan-

2 P. Gokal and E. A. Thompson, Jr., manuscript in preparation.
3 The abbreviations used are: TFIC, transcription factor RNA polymerase I, phosphocellulose fraction C; TK, thymidine kinase; rDNA, DNA encoding rRNA.
4 P. Mahajan and E. A. Thompson, Jr., manuscript in preparation.
Glucocorticoid Inhibition of Gene Expression

A series of experiments has been carried out to study glucocorticoid-mediated inhibition of gene expression in P1798 cells. Three major questions have been defined. (a) What is the mechanism that accounts for this inhibition? (b) To what extent is this inhibitory mechanism similar to that proposed for glucocorticoid-mediated stimulation of transcription? (c) What is the relationship between inhibition of gene expression and inhibition of proliferation? The experiments discussed below address the first of these questions (namely, the mechanism). Regulation of two prototypic genes will be described (those encoding the precursors for rRNA and TK mRNA).

Glucocorticoid Inhibition of Transcription of rDNA. Dexamethasone causes a rapid inhibition of rRNA synthesis in P1798 cells (Fig. 3). Nuclear run on transcription data indicate that this is due to a decrease in the number of RNA polymerase I molecules bound to the nucleolar chromatin and engaged in transcription of rDNA. This is illustrated by the nuclear run on transcription data shown in Fig. 4. Consistent with the pulse-labeling data shown in Fig. 3, nuclear run on transcription of rDNA decreases by ~50% within 6 h. Greater than 98% inhibition is observed within 24 h. The process is completely reversible. If cells are exposed to dexamethasone for 24 h and then transferred to medium without dexamethasone, 60–80% recovery of transcriptional activity is detected within 6 h. Glucocorticoids do not inhibit transcription of 5S RNA genes (Fig. 4).

Initiation of transcription of rDNA has been assayed in nuclei isolated from control and dexamethasone-treated P1798 cells (12). The protocol involves quantitative S1 nuclease mapping of nascent transcripts that are 5' end labeled by transcription in vitro in the presence of [α-thi]ATP. The data indicate that glucocorticoids inhibit the extent to which initiation of transcription can occur.

Hypotonic lysates (S100) were prepared from mid-log phase P1798 cells and from cells that had been exposed to 0.1 μM dexamethasone for 24 h. These were used to transcribe the clone mouse rDNA gene in vitro (7). In these experiments, the template prMAB was digested with PvuII to introduce a double stranded break at a position 292 base pairs downstream from the origin of transcription. Authentic initiation upon this template yields a run off transcript of 292 nucleotides. As shown in Lane a of Fig. 5, S100 extracts from control cells transcribe cloned rDNA with high efficiency. Extracts from dexamethasone-treated cells exhibit little or no ability to transcribe rDNA in vitro (Fig. 5, Lane c). Mixtures of control and hormone-treated extracts (carried out in template excess) are capable of transcribing to a greater extent than that exhibited by control extracts alone (Fig. 5, Lane b). When mixing experiments are carried out in the presence of limiting template, the activity of the extracts is additive (13).

The observation that the hormonal effect prevails in vitro argues against mechanisms that require modification of chromatin. The mixing experiments indicate that extracts from thymine:guanine phosphoribosyltransferase, dihydrofolate reductase, and phosphoglycerate kinase (5).

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complexes (•). In parallel, the ability to form such complexes was assayed using extracts from hormone-treated cells plus highly purified TFIC (O). (8). Extracts from hormone-treated cells lack the ability to form heparin-resistant complexes. In parallel, the ability to form such complexes was assayed using extracts from hormone-treated cells plus highly purified TFIC (O).

hormone-treated cells do not contain a diffusable substance that inhibits transcription or degrades the products of transcription. These observations argue against any mechanism that requires induction of a repressor or direct repression of the rRNA promoter by the glucocorticoid receptor. Furthermore, the synergistic effect observed in Lane b of Fig. 5 suggests that extracts from hormone-treated cells are depleted of a substance that is present in excess in control extracts. Subsequent studies have confirmed this hypothesis. Extracts from control cells have been resolved by chromatography and the fractions were assayed for the ability to reconstitute extracts from dexamethasone-treated cells. A protein with such an activity has been identified. Using the convention proposed by Mishima et al. (14) this protein has been designated as belonging to the “C” class, denoting the observation that the activity elutes from phosphocellulose in a step containing 600 mM KCl. This protein is therefore called TFIC.

TFIC has been highly purified. The thermal lability and chromatographic properties of this entity indicate that it is a protein. The activity copurifies with RNA polymerase I through the early stages of purification, suggesting that the protein is tightly associated with the polymerase. The activity can be resolved from RNA polymerase I and the most highly purified preparations are devoid of detectable RNA polymerase I activity or subunits. TFIC does not bind DNA with high affinity or specificity and is not required for formation of stable, preinitiation complexes (4). However, TFIC is required for formation of heparin-resistant initiation complexes in vitro (8). This is illustrated by the data shown in Fig. 6. Extracts from hormone-treated cells cannot form heparin-resistant “initiated” complexes when incubated with the mouse rDNA promoter (Fig. 6, closed circles). However, the ability to form such complexes is restored by addition of highly purified TFIC (Fig. 6, open circles).

The data indicate that glucocorticoids regulate the amount or activity of a rDNA transcription initiation factor. The mechanism that accounts for this regulation is currently under investigation. The working hypothesis states that proliferative P1798 cells contain high levels of TFIC activity. Upon exposure to dexamethasone, the amount or activity of this factor decreases rapidly. If one assumes that glucocorticoids regulate the synthesis of TFIC, then one must predict that this activity exhibits a short biological half-life. This is indeed the case, as illustrated by the data shown in Fig. 7. Brief exposure to inhibitors of protein synthesis causes rapid inhibition of transcription of rDNA. In this experiment, the ability to transcribe cloned mouse rDNA and the Syrian hamster SS RNA gene was assayed in vitro. Both activities are extremely sensitive to inhibition of protein synthesis (15). Transcription of rDNA, but not of the SS RNA gene, can be reconstituted by addition of partially purified TFIC (15).

Glucocorticoid regulation of rRNA synthesis in P1798 cells constitutes a paradigm for secondary inhibition of transcription by steroid hormones. In this case, transcription is regulated by changes in the amount or activity of an initiation factor and not by direct interaction between the proximal locus (rDNA) and the hormone-receptor complex. The working hypothesis proceeds that the hormone-receptor complex interacts directly with a limited number of primary loci. Some of these encode trans-acting regulatory proteins (e.g., TFIC) which influence the expression of secondary loci (e.g., rDNA). It should be noted that this hypothesis proposes a cascade mechanism whereby a primary interaction may regulate the expression of multiple secondary loci.

The hallmarks of this hypothetical regulatory mechanism are as follows: (a) Regulation should be affected at the level of transcription. Both stimulatory and inhibitory effects can be envisioned. (b) Regulation should be slow, relative to primary responses such as mouse mammary tumor virus. (c) Regulation must be mediated by a protein of short biological half-life. Consequently, genes that are regulated in this hypothetical manner should also be sensitive to inhibition of transcription by inhibitors of protein synthesis. Experiments have been initiated to identify genes that are transcribed by RNA polymerase II and regulated in this manner. One candidate for such a mechanism is described below.

Glucocorticoid Inhibition of Transcription of the Gene Encoding Thymidine Kinase. Glucocorticoids inhibit the incorporation of exogenous [3H]thymidine and cause a decrease in the activity of thymidine kinase in P1798 cells (5). The abundance of TK mRNA decreases, roughly in parallel with thymidine kinase activity. Approximately 50% reduction in TK mRNA abundance is observed at 12 h, as shown in Fig. 8 (see also Fig. 3). Nuclear run on transcription data (Fig. 9A) indicate that regulation under these circumstances is affected by a decrease in the number of RNA polymerase II molecules engaged in transcription of the TK gene. Inhibition of transcription parallels de...
Thymidine Kinase mRNA

Dex 0 12 24 Hr
TK

18S
1
2
3

Fig. 8. Glucocorticoid regulation of the abundance of mRNA encoding thymidine kinase. Total RNA was extracted from mid-log P1798 cells and from those treated with dexamethasone (DEX) for 12 or 24 h. RNA was resolved by agarose gel electrophoresis under denaturing conditions and abundance of TK mRNA was determined by hybridization to a nick-translated complementary DNA fragment. The abundance of 18S RNA was measured as an internal control for RNA degradation and transfer.

As shown in Fig. 9B, inhibition of protein synthesis for 2 h causes ~90% inhibition of nuclear run on transcription activity. Nuclear run on transcription of the 5S RNA gene was inhibited ~65% in this experiment.

Thymidine kinase therefore fulfills the hypothetical criteria for a gene that is regulated via glucocorticoid-mediated changes in the activity of one or more transcription factors. Regulation appears to be secondary (although this remains to be directly tested). Transcription of TK appears to require a protein of short biological half-life, which may be regulated by glucocorticoids. Many aspects of this hypothesis are untested, but experiments toward this end are currently in progress. Nevertheless, the gene encoding thymidine kinase appears to be a reasonable candidate for secondary inhibition by a mechanism analogous to that described for rDNA.

Posttranscriptional Regulation by Glucocorticoids. Glucocorticoids regulate the amount or activity of transcription initiation factors which, in turn, determine the rate of transcription of rDNA and perhaps other genes. Our original hypothesis proposed that hormones regulate the rate of synthesis of these proteins, most likely at the level of transcription of the cognate genes. We have recently come to reevaluate this hypothesis and to consider a possible posttranscriptional mechanism for regulation of synthesis of transcription factors. The basic observations leading to this revised hypothesis were obtained from studies of hormonal regulation of expression of genes encoding ribosomal proteins.

Glucocorticoids inhibit the synthesis of rRNA. Synthesis of ribosomes is therefore precluded. Experiments were undertaken to determine if synthesis of ribosomal proteins was coordinately regulated in P1798 cells. It was initially determined that dexamethasone did not affect the abundance of mRNAs encoding ribosomal proteins L32, L18, L7, L30, S16, or L19 (9). However, translation of these mRNAs was severely restricted in dexamethasone-treated P1798 cells. This was ascertained by analysis of polysomal distribution of mRNAs. Polysomes from control and steroid-treated cells were resolved by rate zonal centrifugation on sucrose density gradients. Fractions were collected and polysome distribution was determined by measuring total RNA concentration. Distribution of the mRNAs of interest was determined by blot hybridization. Using this approach, it is possible to determine if a particular mRNA is associated with polysomes and therefore undergoing translation. Those mRNAs that resolve in gradient fractions of less than polysomal velocity are considered to be untranslated. Representative data are shown in Fig. 10. The upper panel illustrates the distribution of actin and ribosomal protein L18 mRNAs from control cells. The vertical bars above fraction 7 indicate the division between monoribosomes or smaller (fractions 7–12) and polyribosomes (fractions 1–6). Approximately 90% of actin mRNA is associated with polysomes (average >10 ribosomes/374-codon mRNA). The mRNA encoding ribosomal protein L18 is translated with somewhat lower efficiency than that observed for actin mRNA. Approximately 70% of L18 mRNA is associated with polysomes (average >10 ribosomes/374-codon mRNA) in control cells. Dexamethasone causes a significant redistribution of L18 mRNA (lower panel). Approximately 20% of this mRNA is associated with polysomes in this sample (fractions 1–7). Nevertheless, actin mRNA is not significantly affected (~86% polysomal). Similar results were obtained with the other ribosomal protein mRNAs, excepting L32 mRNA.

The effects of dexamethasone are reversible, as shown in Fig. 11. In this case, cells were treated with dexamethasone for 24
h. Thereafter, the hormone was withdrawn and the polysomal distribution of superoxide dismutase mRNA and ribosomal protein L30 mRNA was determined as a function of time. About 20% of L30 mRNA is associated with polysomes in hormone-treated cells. Following withdrawal, a rapid recruitment of L30 mRNA occurs and control levels of translation are achieved within ~6 h. Translation of superoxide dismutase mRNA is also inhibited to a slight extent and one observes reversal upon removal of dexamethasone.

The salient features of this phenomenon may be summarized as follows. (a) Glucocorticoids have a subtle effect upon protein synthesis in P1798 cells. In untreated cells, ~60% of the ribosomes exist as polysomes. After 24 h exposure to dexamethasone, ~45% of the ribosomes exist as polysomes (9). This is associated with <20% inhibition of incorporation of [3H]leucine. (b) Some mRNAs are translated in hormone-treated cells with an efficiency similar to that observed in control cells. It is probably significant that these mRNAs are translated with very high efficiency (>90% polysomal) in control cells. (c) A second class of mRNAs is excluded from polysomes, and therefore presumably untranslated, in glucocorticoid-treated cells. One should note that such mRNAs are translated with relatively low efficiency (<75% polysomal) in control cells. Although the data are limited to ribosomal protein mRNAs, one may assume that these are representative of a larger class of mRNAs that is poorly translated under optimum conditions. It is proposed that glucocorticoids reduce the efficiency of translation in P1798 cells. Under these circumstances, mRNAs with high affinity for ribosomes (e.g., actin mRNA) remain to be translated with near control efficiency. However, mRNAs with weak affinity for ribosomes (e.g., ribosomal protein mRNA) are disproportionately inhibited.

Discussion

The prevailing hypothesis states that all of the actions of glucocorticoids are mediated by the glucocorticoid receptor. This protein is among the most extensively studied mammalian transcription factors, and a considerable amount of information is available concerning the mechanism whereby the hormone-glucocorticoid receptor complex stimulates transcription of a number of target genes. Less is known concerning inhibition of gene expression by glucocorticoids. By analogy with the mechanism that accounts for stimulation of transcription, one may propose that the receptor may function as a repressor (16). Binding of the hormone-receptor complex may inhibit initiation of transcription, perhaps by displacing essential transcription factors (20, 21). Although direct repression remains to be demonstrated, the mechanism provides a testable hypothesis to account for primary inhibition of transcription by glucocorticoids.

Secondary inhibition of transcription, as involved in regulation of expression of rDNA and perhaps thymidine kinase, is certain to involve more complicated mechanisms. The simplest mechanism that one might envision would be direct, glucocorticoid-mediated stimulation of a gene encoding a trans-acting repressor. This mechanism is logically compelling, but it does not appear to account for glucocorticoid inhibition of transcription of rDNA or any other gene studied to date.

Glucocorticoids cause a decrease in the amount or activity of an essential RNA polymerase I transcription initiation factor. At this time, it is unclear if this is due to inhibition of synthesis of the factor or to inactivation. Inactivation could be due to induction, by the steroid, of an enzyme that modifies the factor. Inhibition of synthesis could be due to receptor-mediated repression of the gene that encodes the factor. These are relatively simple mechanisms, and no data preclude either possibility.

Translational control of the synthesis of transcription factors is obviously a more complex mechanism. The working hypothesis predicts that there exists within cells a subset of mRNAs that is translated with relatively poor efficiency. When the overall translational activity of cells is diminished, these mRNAs are at a competitive disadvantage and are disproportionately inhibited. It is proposed that some of these mRNAs encode trans-acting factors of short biological half-life. These govern the expression of a number of genes that are involved in regulation of proliferation or maintenance of the proliferative state.

The physiological significance of such a mechanism is clear.
The experiments describing translational control of ribosomal protein central role in almost all of the projects undertaken in this laboratory. Alice Cavanaugh initiated studies on TFIC. Dr. Preeti Gokal has of the regulatory mechanism remain to be resolved for this or...of concentrations, as originally described by Munck (17), Nordeen and Young (18), and coworkers.

In conclusion, we submit that secondary regulation, of the sort described above, plays an important role in physiological regulation by steroids. The working hypothesis proposes that such hormones interact with a limited number of primary loci. Among these are genes the products of which act in trans to regulate the expression of distal loci. The rDNA transcription factor TFIC is a paradigm for such a mechanism. The details of the regulatory mechanism remain to be resolved for this or any other such transcription factor. Several alternative pathways may be envisioned and one may only speculate at this time. The salient feature of this hypothetical mechanism is that it proposes a cascade in which transcription factors function as second messengers. These serve to amplify the hormonal response and to orchestrate complex changes in the pattern of gene expression that are associated with physiological transitions such as changes in mitotic activity.

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