Hormone Withdrawal Triggers a Premature and Sustained Gene Activation from Delayed Secondary Glucocorticoid Response Elements*

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Glucocorticoid regulatory elements, denoted GREs and delayed secondary GREs (sGREs), bind the purified glucocorticoid receptors via distinctive sequence motifs and confer a primary and delayed secondary hormone inducibility, respectively, upon a linked reporter construct in stably transfected mammalian cells. The delayed secondary responses, but not the primary responses, are preceded by a time lag of several hours and blocked by protein synthesis inhibitors. In this report, we further characterized and distinguished these hormonal inductions. A 206-base pair DNA fragment from the hepatic rat α2-globulin (RUG) gene, containing at least two delayed sGREs, was specifically activated by glucocorticoids in a dose-dependent manner via a process which is sensitive to receptor antagonist RU486. Delayed sGRE-stimulated production of correctly initiated transcripts was preceded by a time lag of 2 h, a time when the GRE-mediated induction had reached maximal levels. A pulse of glucocorticoids sustained maximal activation of the delayed secondary response but not the primary response. In fact, hormone withdrawal triggered a premature induction of this delayed secondary response, suggesting that delayed sGREs are under both negative and positive control of the hormone receptor. Two separable elements of the 206-base pair fragment, including the 29-base pair sequence of a single receptor binding site, activated the reporter expression as effectively with transient, pulsatile exposure to hormone as with continuous exposure. Our results suggest that the information content of a hormonal pulse is retained, or “memorized,” more persistently by a receptor binding site of delayed sGREs than those of the prototypical GREs.

Upon binding hormone, steroid receptor proteins bind productively to a number of primary-response genes and rapidly stimulate or repress their transcriptional rates. For instance, in the case of glucocorticoid receptors, the DNA sequences near and/or within many hormone responsive genes selectively bind glucocorticoid receptors in vitro and act as receptors.

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The abbreviations used are: GRE, positive primary glucocorticoid response element; sGRE, secondary GRE; RUG, rat α2-globulin; CAT, chloramphenicol acetyltransferase; TK, thymidine kinase; dex, dexamethasone; H-R, activated hormone receptor complex; pRP, a dexamethasone-inducible primary response protein; R², the glucocorticoid receptor of hormone-withdrawn cells.
mammalian delayed secondary responses have been recognized (8-13). For instance, the hepatic rat α2-globulin (RUG) genes are transcriptionally regulated by glucocorticoids (14) in a manner characteristic of delayed secondary genes (15-17). We recently reported that purified glucocorticoid receptors bind selectively at multiple sites (18) via unusual sequence motifs to segments of RUG-transcribed DNA (19). Our findings suggest that some of the receptor binding sites detected in vitro are components of a timing element, i.e. a “clock,” for delayed gene activation in cultured cells. First, DNA fragments containing 1.5 to 5 receptor footprints from this region confer hormonal responsiveness upon a linked reporter construct in stably transfected cell lines. The induction of the reporter is preceded by a time lag and is blocked by inhibitors of protein synthesis. Second, the RUG DNA fragments that more efficiently bind to the receptors appear to be stronger inducers. Third, the DNA sequence of a single receptor footprint is a delayed sGRE in stably transfected cells (19).

We showed the following in the present study: (i) the hormonal response mediated by the RUG’s delayed sGREs, but not by GREs, is maximally activated by a pulse of glucocorticoids and prematurely induced by hormone withdrawal in mammalian cells; (ii) importantly, transient or continuous hormonal treatments are similarly efficient in the mediation of a secondary response by the sequence of a single receptor footprint.

MATERIALS AND METHODS

Cultured monolayers of the transfected mouse mammary Mm5MT cells were grown and maintained in media supplemented with 4% growth hormone-deficient bovine serum or with 5% fetal bovine serum as described (18, 19). Both sera were “defined” and were chosen to be particularly low in steroid and thyroid hormone content (5 × 10⁻¹⁰ to 10⁻¹¹ M; Hyclone). Unless noted otherwise, the steroid and thyroid hormones (Sigma) were added to the growth media at a final concentration of 5 × 10⁻⁷ M, RU486 at 1.0 × 10⁻⁸ M, and growth hormone at 2.5 × 10⁻⁸ M. RU486 and bovine growth hormone were generous gifts of Roussel-UCLAF, Romainville, France and Monsanto, St. Louis, respectively. Previous experiments (18, 19) established that the induction of CAT activity is associated with the stimulation of the relative levels of the correctly initiated TK.CAT promoter activity and slightly lower fold inductions were observed with cells grown in media supplemented with bovine serum relative to fetal bovine serum. The duration of time lag was essentially the same under both culture conditions. For all experiments reported, similar results were obtained in two to six additional experiments involving the same or different clonal isolates and/or transfected population. Hormone withdrawal: after initial pulse with dex (Figs. 2-8), cells were rinsed three times with 5 ml each of wash media over a course of 5 min and grown in hormone free media for 24 h from the time of hormone addition. Wash media, previously equilibrated at 37 °C in a humid atmosphere of air/CO₂ (92:8), was the complete growth media lacking added dex. The basal promoter activity was not significantly altered over the course of experiments or affected by the rinsing procedure. Similarly, the rinse procedure did not substantially affect the fold induction of CAT activity in extracts from cells that had been continuously exposed to dex for 2 or 24 h. All other methods were performed as described (18, 19).

RESULTS

Glucocorticoid Hormone-specific Activation of a Delayed Secondary Response—Pituitary ablation-hormone replacement studies have identified hormones that induce glucocorticoids, growth hormone, androgens, and thyroid hormones) and repress (estrogen) the transcription of the hepatic RUG genes (14, 20-23). An aspect of this complex multihormonal regulation that has been mimicked in culture is the delayed secondary response to glucocorticoids (15-18). This response is mediated by DNA sequences near or within the RUG genes (−240 to +3865 in Fig. 1A) (17). Within this region, the 2065-bp SB (RUG2065, −240 to +3865) and 206-bp SV (RUG206, +1800 to +2006) restriction fragments (Fig. 1A) are most active in conferring a delayed secondary glucocorticoid responsiveness upon a linked TK.CAT construct (OTCU, Fig. 1B) in stably transfected cells (UTC02065 and UTCU206, Fig. 1B) (18, 19).

Hormonal requirements for the regulation of delayed secondary steroid hormone response elements have not been previously characterized under defined culture conditions. Given the complexities suggested by animal experiments, we examined whether the reporter activation from RUG DNA fragments is specific to glucocorticoids in the cell lines UTC02065 and UTCU206. As expected, CAT activity was inducible by dexamethasone (dex) in UTC02065 and UTCU206 cells (Fig. 1D, D, open and closed bars) but not in the negative control cells UTCO (Fig. 1D, D, dotted bars) or OTCO (below; 18, 19). In contrast, under the present conditions, CAT activity was not significantly affected by estrogen (E), progesterone (P), testosterone (T), thyroid hormone (T3), or growth hormone (G) in UTC02065 cells, UTCU206 cells, or OTCA cells (Fig. 1D). In addition, the induction of CAT activity by dex was not affected by the continuous co-presence of estrogen, progesterone, or growth hormone (Fig. 1D). Moreover, the specificity of induction by dex was demonstrated by the use of the glucocorticoid receptor antagonist RU486 (24, 25). This agent blocks dex-induced CAT activity and has no agonist activity at the concentrations used (R and DR, Fig. 1D).

Based on previous studies with mammary tumor virus (26) and with RUG genes (15-17), we anticipated that dex dose-response curves for the induction of primary and secondary responses in mammalian cells would extend over ~2 orders of magnitude and become maximal at ~0.1 to 1.0 × 10⁻⁶ M dex. As a marker for primary response, we turned to OTCS cells, where TK.CAT activity is under the control of linked GREs (Fig. 1B) (18). As expected, the dex dose-responses for induction of CAT activity are similar in the TK.CAT cells, UTC02065 cells, and UTCO206 cells. Greater than 80% of induction occurred between 1.0 × 10⁻³ and 1.0 × 10⁻⁷ M dex, and half-maximal induction required between 2 × 10⁻⁶ and 5 × 10⁻⁶ M dex (Fig. 1C). In the present context, our results suggest that activation of delayed sGREs in transfected Mm5MT mammary cells is specific to glucocorticoids and is likely to be mediated by the glucocorticoid hormone receptor.

Withdrawal from Glucocorticoids Triggers a Premature Activation of Delayed sGREs—We examined whether a short pulse of dex is as effective as continuous hormonal treatment for induction of CAT activity from GREs and delayed sGREs. Our experimental strategy is schematized in Fig. 2A. The cells were pulsed with dex for periods of 5, 15, 30, 45, 60, or 120 min and then withdrawn from dex for the rest of the 24-h period from the time of hormone addition (see “Materials and Methods”). CAT activity was induced by dex in UTC02065 cells (Fig. 2B) and in GTCS cells (Fig. 2C). In both cell lines, the magnitude of induction is a function of the initial duration of exposure to the hormone (Fig. 2D, cf. curve B with curve C). In GTCS cells, a dex pulse of 2 h induces 20% to 30% the CAT activity of a continuous 24-h exposure to hormone (Fig. 2D, curve C). In striking contrast, equivalent amounts of CAT activities are induced in the UTC0206 cells treated with dex

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For 2 h and withdrawn from hormone for 22 h or treated continuously with dexamethasone (Fig. 2D, curve B).

The kinetics of induction of CAT activity in GTCS and OTCU206 cells exposed to hormone for 2 h were compared with cells grown continuously in dexamethasone throughout the course of the experiment (Fig. 3). In GTCS cells, CAT activity was induced to a greater extent in the continuous presence of dexamethasone (Fig. 3A and curve A in E) as compared to a 2-h exposure to hormone (Fig. 3B and curve B in E). In contrast, in OTCU206 cells, withdrawal from dexamethasone after a 2-h hormonal pulse leads to a premature induction of CAT activity (Fig. 3D and curve D in Fig. 3F) when compared to cells grown in the continuous presence of dexamethasone (Fig. 3C and curve C in F). There is a reduction of ~2 h in the time lag that precedes induction (cf. curves C and D in Fig. 3F). Maximal induction is achieved at an earlier time point in withdrawn cells (Fig. 3F, curve D) than in cells continuously exposed to dexamethasone (Fig. 3F, curve C). Furthermore, CAT activity is induced to approximately the same extent under both conditions (Fig. 3F and not shown).

The data presented in Fig. 2 and Fig. 3 could imply that the induction of CAT RNA continues in the hormone-with-
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![Diagram](image)

**Fig. 3.** Hormone withdrawal triggers premature activation of delayed sGREs. A and C, autoradiograms show relative CAT activity in GTCS.C12 cells (A) and OTCU206.1.C6 cells (C) grown continuously in the presence of dex for the indicated times. B and D, autoradiograms show relative CAT activity in GTCS.C12 cells (B) and OTCU206.1.C6 cells (D) pulsed with added dex between 0–2 h and harvested, or rinsed with wash media as in Fig. 2, and their growth continued in wash media over the indicated time course between 2 h and 16 h from the time of dex addition. E and F, quantitation of data in A–D is represented by curves A–D, respectively; other symbols are as in Fig. 1.

drawn OTCU206 cells but not in the GTCS cells. Alternatively, it is possible that OTCU206 cells support a higher level of induction than the GTCS cells. Assays of CAT activity do not distinguish between these possibilities, in part, because the turnover of CAT activity in the mammary cells is not detectable during the indicated course of the experiments. In contrast, the turnover of CAT RNA is measurable under our assay conditions. Therefore, to directly explore the dynamics of induction/withdrawal, we monitored the production of correctly initiated TK:CAT RNA in GTCS and OTCU206 cells. Fig. 4A and curve A in C show that continuous exposure of GTCS cells to dex resulted in a rapid induction of TK:CAT transcripts and a 50% maximal induction after 15 min. However, in GTCS cells that were exposed to dex for 1 h and subsequently withdrawn from hormone, CAT RNA transcripts declined rapidly (Fig. 4A, curve AW in C). In contrast, in continuously dex-exposed OTCU206 cells, the induction of TK:CAT transcripts at a slower rate than in GTCS cells (Fig. 4B, curve B of C). Strikingly, in every case, OTCU206 cells pulsed with dex for 1 h and, when withdrawn from hormone, demonstrated a premature induction of transcript production (Fig. 4C; cf. curve B with curve BW in Fig. C). Moreover, distinct from GTCS cells, correctly initiated TK:CAT transcripts are produced to the same extent in hormone-withdrawn OTCU206 cells and in OTCU206 cells continuously
exposed to hormone (Fig. 4C; cf. curve B with curve BW).

Our experiments presented here and elsewhere (18, 19) document certain discordance between CAT activities and CAT RNA transcript levels. This applies to the GRE as well as the delayed sGRE-linked reporters. For example, an 10-to 20-fold induction of CAT activity (Fig. 3, E and F) corresponds to an ~3.5- to 4-fold stimulation of correctly initiated CAT RNA transcripts (Fig. 4C). Similarly, the premature induction of CAT activity is far more pronounced than that of CAT RNA (cf. curves C and D in Fig. 2F with curves B and BW in Fig. 4C). In addition, the duration of the time lag is 6 and 8 h by CAT assay (Fig. 3F) and 1.5 and 2 h at RNA level (Fig. 4C). We have repeated these experiments 2 to 6 times and, in every case, obtained similar results. We suspect that the observed discordance may have at least two causes. (i) Given that CAT activity is much more stable than CAT RNA, small differences at the RNA level are likely to be amplified to larger inductions in CAT activity. (ii) The generation of CAT activity from CAT RNA transcript is an inefficient process in the present contexts. Importantly, however, the conclusions resulting from both types of analyses are in general agreement. The relative instability of CAT RNA appears to have been advantageous in allowing us to further distinguish between the primary and the secondary responses. Relative Efficiencies of Transient and Continuous Hormonal Signals for Gene Activation from a Single Glucocorticoid Receptor Footprint Site—Previous results suggested that multiple delayed sGREs reside on the 206-bp SV fragment of RUG DNA (19). Here, we tested whether multiple delayed sGREs are essential for efficient gene activation by a transient hormonal signal and whether the delayed sGRE contained within the sequence of a single receptor footprint is sufficient for maximal response to a pulse of glucocorticoids. The two separate components of the 206-bp fragment tested are a 131-bp HaeIII/PvuII (HV) restriction endonuclease fragment (containing at least two receptor footprint sites) and a 29-bp element corresponding to the sequence of a footprint site for purified glucocorticoid hormone receptor (Fig. 5A).

The cell lines OTCU131, UTC029, OTCA, and OTCO (Figs. 1B and 5B) were grown either in the continuous presence of added dex or exposed to hormone for 2 h and followed by 22 h of hormone withdrawal. As expected, neither regimen affected CAT activity in OTCA cells or in OTCO cells (Fig. 5C; 1.0- to 1.5-fold). In contrast, in hormone-withdrawn OTCU131 and UTC029 cells, CAT activity was induced 6- to 8-fold and 5.5- to 7-fold, respectively. Notably in these cell lines, hormone regulated expression was nearly identical, whether or not cells were withdrawn from dex (Fig. 5C).

**DISCUSSION**

Biological clocks and other context-related regulatory phenomena strongly influence the level of circulating hormones in vertebrates. Generally, a primary response requires the continuous presence of a high level of hormone (5; for notable exceptions see Refs. 27-29). How are the delayed temporal effects of steroid hormones propagated amid fluctuations in hormone levels that in many instances may decrease well below the $K_d$ for receptor? Interestingly, experiments with *Drosophila* show that temporal coordination of developmental and physiological responses to steroid hormones is driven by at least two classes of delayed secondary genes: those which are maximally induced in hormone-withdrawn cells, and those which are induced only in cells continuously treated with the hormone (5). Perhaps both of these regulatory network types occur widely in vertebrates as well.

We showed the following in this report. (i) A 206-bp RUG transcribed DNA fragment, containing at least 2 delayed sGREs and 5 glucocorticoid receptor binding sites (18, 19), is prematurely activated by hormone withdrawal (Figs. 3F and 4C). Importantly, the lag period was reduced but not abolished. (ii) The delayed sGREs are similarly active in cell lines treated transiently or continuously with the hormone (Figs. 2D, 3F, 4C, and 5C). The delayed sGRE-mediated hormonal response was specific to glucocorticoids (Fig. 1D), and its magnitude was dependent on the dosage (Fig. 1C) and the duration of hormonal treatment (Fig. 3F). Further, the optimal duration of a hormonal pulse (~45-120 min; Fig. 2D) did not exceed the length of the time lag (Figs. 3F and 4C). In contrast, a mammalian GRE-mediated primary response was not prematurely induced, and its maximal or sustained activation was dependent on the continuous presence of the hormone (Figs. 2D, 3E, and 4C). (iii) Maximum responsiveness to a transient hormone pulse is indicated by a single receptor binding site (RUG29 in Fig. 5C), suggesting that the RUG DNA sequences residing outside of the receptor footprints are not essential. Further, the pulsed or continuous
hormone-exposure responses are similar for delayed sGREs containing 1, 2, or 5 receptor binding sites. Thus, cooperation between receptor binding sites does not appear to contribute to this similarity.

These results suggest that, in addition to their positive role as an inducer, glucocorticoids also negatively affect the delayed sGRE activity. Negatively, because hormone is involved in imposing a time lag as evidenced by a shortened time delay upon hormone withdrawal. Further implications are that the delayed secondary response is competitively inhibited during the time lag and that induction represents the reversal of this inhibition, possibly by certain factors that accumulate during the course of hormone treatment. Moreover, the results demonstrate that a hormonal pulse is memorized longer by the receptor binding sites of delayed sGREs than those of GREs. Therefore, the hormone- and time-dependent genetic alterations that occur within a 1-h hormonal pulse are sufficiently stable to survive the hormone withdrawal and can be utilized at a delayed sGRE but not at GREs (Fig. 4C).

How could these genetic alterations be "retained" by delayed sGREs but not by GREs? In the consensus GREs, the two receptor binding half-sites are inverted pseudo-repeats and separated by 3 bp (1–3). This arrangement of half-sites appears important for their cooperative occupancy by a receptor dimer (30–32), for presentation of activation surfaces of receptor to the transcriptional components (33, 34), and for the GRE activity (9, 35). In contrast, the arrangement of the receptor binding half-sites of the delayed sGRE is heterogeneous and differs from that of the consensus GRE. In the 29-bp RUG element, for example, the half-sites are direct pseudo-repeats separated by 6 bp (19). It is possible that the reason why this element acts as a delayed sGRE is because it binds to the receptor inefficiently and the bound receptors are poised differently than those bound to GREs (19). Perhaps delayed sGREs of the RUG DNA are occupied only when the receptor is oligomerized with certain auxiliary factors. Since the receptor affects the delayed sGRE negatively during the time lag and positively during the inductive phase, distinct auxiliary factors might be responsible for receptor-sGRE interactions. Receptor binding could rely on one constitutive auxiliary factor initially and on another one that accumulates during the time lag later. We infer that such receptor-factor oligomers cannot productively bind to GREs. We propose that the delayed sGREs, but not the GREs, are able to retain this information and efficiently propagate the genetic consequences of a hormonal pulse without strict dependence on the presence of hormone.

Models for Regulation by Delayed sGREs—Our results summarized above are not readily compatible with models (cascade or more complex) that assign either a strictly positive or negative regulatory role to the delayed sGREs. For example, according to a negative regulatory model, we could envision that a product of a primary gene may interact with delayed sGREs and repress its activity in the absence of the hormone. Upon hormonal treatment, the activity of such a factor could be down-regulated and thereby lead to induction of a secondary response. To account for the ability of protein synthesis inhibitors to block secondary responses, such a factor would have to be a member of that class of proteins that is stabilized by the same inhibitors (for an example see Ref. 36). Instead, we believe that our data support and extend the model proposed by Ashburner and his colleagues (5; see the introduction to the text). However, it may be instructive to consider another simple model, also consistent with our data, but differing substantially from that proposed for the ec dysone-regulated late puff (4, 5).

According to this speculative proposal, the hormone-receptor complex (H-R) would inductively function at delayed sGREs only by interacting with both specific DNA sequences and an auxiliary protein. This auxiliary protein, termed secondary response protein (pRP), is induced in a GRE-dependent manner and must accumulate to a threshold level before a delayed secondary response can be activated. Thus, a delayed sGRE remains inactive under the conditions where the ratio of the H-R-pRP complex to H-R is low, i.e. during the time lag and when accumulation of pRP is blocked by protein synthesis inhibitors. In this case, H-R in combination with another cellular factor (F) competitively inhibits the H-R-pRP binding to delayed sGREs (Fig. 6; +Hearly). In contrast, at higher H-R-pRP to H-R ratios (during the inductive phase), the binding site occupancy by an H-R-pRP oligomer is favored (Fig. 6; +Hlate). Further, in cells withdrawn from a hormonal pulse, some pRP has already been accumulated, and H-R is converted to unliganded hormone-withdrawn receptors (R). In these cells, the R-pRP oligomers, by effectively competing with the R-F complex for delayed

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*G. Chan and F. Payvar, unpublished observations.*

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Fig. 6. A model for the activities of delayed sGREs. Proposed is a collaborative competition model for the RUG DNA-mediated hormonal response which is preceded by a time lag, blocked by inhibitors of protein synthesis, prematurely induced by hormone withdrawal, and induced with similar efficiency by transient or continuous treatment with glucocorticoids. Three states are depicted: early after hormone treatment (+Hearly), late after continuous hormone treatment (+Hlate), and late after withdrawal from a hormonal pulse (+Hlate). In each panel, the different size arrows depict the relative activity of a transcriptional unit. Delayed sGREs are denoted by a solid line internal to arrow; dotted lines between sGRE-bound factors and the promoter indicate the repressive (−) and inductive (+) activities. +Hearly, an oligomer of the hormone receptor complex (H-R; arrowhead) and a regulatory factor (F; closed circle) binds to the delayed sGRE and repressively interacts with the transcriptional components. The activity of F need not be affected by hormone. During this period, a protein product of certain primary response genes (pRP) is hormonally induced in a process sensitive to the protein synthesis inhibitors. +Hlate, the pRP (open circle)-H-R oligomer competitively displaces the repressive H-R-F complex from the delayed sGRE and inductively interacts with the transcriptional machinery. +Hlate, an oligomer of the hormone-withdrawn receptor (R; open triangle) and pRP acts as +Hlate. However, since H-R-F is a more effective competitive inhibitor of H-R-pRP binding than is R-pRP, there is a premature induction upon hormone withdrawal. The crucial features of the model are that (i) in order for H-R to act negatively or positively at delayed sGRE, it has to be modified via interaction with factor F and pRP, respectively, and (ii) the receptor binding site of the delayed sGRE, but not the GRE, is occupied productively by H-R-pRP and R-pRP oligomers. The model is an extension of the previous notions on combinatorial regulation of the primary (1, 37, 38) and the secondary transcriptional responses (18, 19, 39).
sGRE binding, cause/maintain induction (Fig. 6; W:late). To account for a premature induction upon hormone withdrawal, H - R - F would have to be a more efficient competitive inhibitor of H - R - pRP binding than is RW - F relative to RW - pRP.

It remains formally possible that the positive (5, 18) and/or negative (18) effects of glucocorticoids on delayed sGREs are mediated indirectly via hormone-regulated nonreceptor factors without direct involvement of the receptor. Notably, the RUG29 element does represent the sequence of a single receptor footprint, does selectively bind the receptor via sites (half-sites) that are GRE-like, and does mediate a delayed secondary response (19). Thus, as the simplest interpretation of our data, we prefer the model proposed in Fig. 6 involving direct interaction of receptor with delayed sGREs. However, as with others in the family of DNA binding proteins that bind weakly to degenerate sequences (37), productive receptor-delayed sGRE interactions will have to involve nonreceptor factors, possibly similar to the kinds already described (40–45). For this and other reasons (19), our model invokes the participation of non-receptor factors (F and pRP in Fig. 6). This model envisions that the trans-activation domain of the receptor can inductively contact the transcriptional components only when the pRP-receptor oligomers are bound to the delayed sGRE.

The receptor-pRP oligomer is proposed to have at least three functional roles. First, to competitively displace the repressive H - R - F oligomers from delayed sGREs. The possibility that a large repertoire of distinct transcription factor complexes (homodimers, heterodimers, and higher order oligomers) may play a regulatory role by competing for the same binding site is compatible with data in several systems (reviewed in Ref. 46). Second, the H - R - pRP and not the H - R - F is a positive regulator of transcription. The pRP could produce a conformational realignment of the trans-activation domain of the receptor, enable the H - R - pRP complex to inductively engage the transcriptional components. Recent data suggest that depending on the biological contexts, activities of the receptor are modified via interaction with differing regulatory factors. This, in turn, appears to dramatically affect the direction, magnitude, and duration of hormonal responses (41–45). Third, our model envisions that the RW - pRP - sGRE complexes are more stable than RW - GRE. This could explain why RUG’s delayed sGREs, but not the GREs, continue to amplify the original hormonal pulse long after hormone has been washed out. The fate of the GRE-bound receptors is a matter for debate (45, 47–51). However, it is intriguing that while the hormone agonist is cleared to the cytoplasm rapidly within the first 1–2 h after hormone withdrawal (52, 53), the RW continue to reside in the nucleus for ~8–12 h (45). The structure and properties of such receptors as well as the biological significance of a sustained nuclear residency of RW are enigmatic. Our model speculates that the nuclear retention of the RW results, in part, from their engagement at certain delayed sGREs or GREs whose activity is not abruptly terminated by hormone withdrawal. In any case, the defined RUG DNA elements and their activities in hormone-withdrawn cells provide advantageous markers for testing the proposed notions in the future.

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