Review

Structural variants of glucocorticoid receptor binding sites and different versions of positive glucocorticoid responsive elements: Analysis of GR-TRRD database

Vasily M. Merkulov[1], Tatyana I. Merkulova[*]

Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Sciences, Novosibirsk 630090, Russia

A B S T R A C T

The GR-TRRD section of the TRRD database contains the presently largest sample of published nucleotide sequences with experimentally confirmed binding to the glucocorticoid hormone receptor (GR). This sample comprises 160 glucocorticoid receptor binding sites (GRbs) from 77 vertebrate glucocorticoid-regulated genes. Analysis of this sample has demonstrated that the structure of only half GRbs (54%) corresponds to the generally accepted organization of glucocorticoid response element (GRE) as an inverted repeat of the TGTTCT hexanucleotide. As many as 40% of GRbs contain only the hexanucleotide, and the majority of such "half-sites" belong to the glucocorticoid-inducible genes. An expansion of the sample allowed the consensus of GRbs organized as an inverted repeat to be determined more precisely. Several possible mechanisms underlying the role of the noncanonical receptor binding sites (hexanucleotide half-sites) in the glucocorticoid induction are proposed based on analysis of the literature data.

© 2009 Elsevier Ltd. All rights reserved.

Contents

1. Introduction

Glucocorticoid hormones are involved in regulation of the main vital processes in the body of vertebrate animals—growth, differen-tiation, reproduction, adaptation, and behavior [1,2]. The glucocor-ticoids mainly act at a transcription level via binding to the intra-cellular receptor of these hormones. The formed hormone–receptor complexes up- or down-regulate the corresponding genes by bind-ing to the recognized DNA regions and/or entering protein–protein interactions with other transcription factors [3–5].

Glucocorticoid receptor (GR) is a ligand-inducible transcrip-tion factor belonging to the nuclear receptor superfamily. The DNA-binding domain of the members of this superfamily contains two loop structures, the so-called zinc fingers, with four cysteine residues coordinated by zinc ions at their base [6–8]. Two hexanu-

* Corresponding author. Laboratory of Gene Expression Control, Institute of Cytol-ogy and Genetics, pr. Lavrenteva, 10, Novosibirsk 630090, Russia.
Tel.: +7 383 333 2860; fax: +7 383 333 1278.
    E-mail address: merkti@noboch.nsc.ru (T.I. Merkulova).
    [1] Address: Laboratory of Gene Expression Control, Institute of Cytology and Genet-ics, pr. Lavrenteva, 10, Novosibirsk 630090, Russia.

(A)   (n = 25) [Beato et al., 1989]

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A	5	15	10	**55**	10	**55**	40	15	20	15	0	5	5	0	5
C	25	0	20	10	**75**	25	25	15	35	0	0	0	25	**100**	10
G	**45**	**65**	20	0	0	10	15	25	25	5	**95**	5	5	0	0
T	25	20	**50**	35	15	10	0	45	20	**80**	5	**90**	**65**	0	**85**

G G T A C A N N N T G T T C T

(B)   (n = 87) [GR-TRRD]

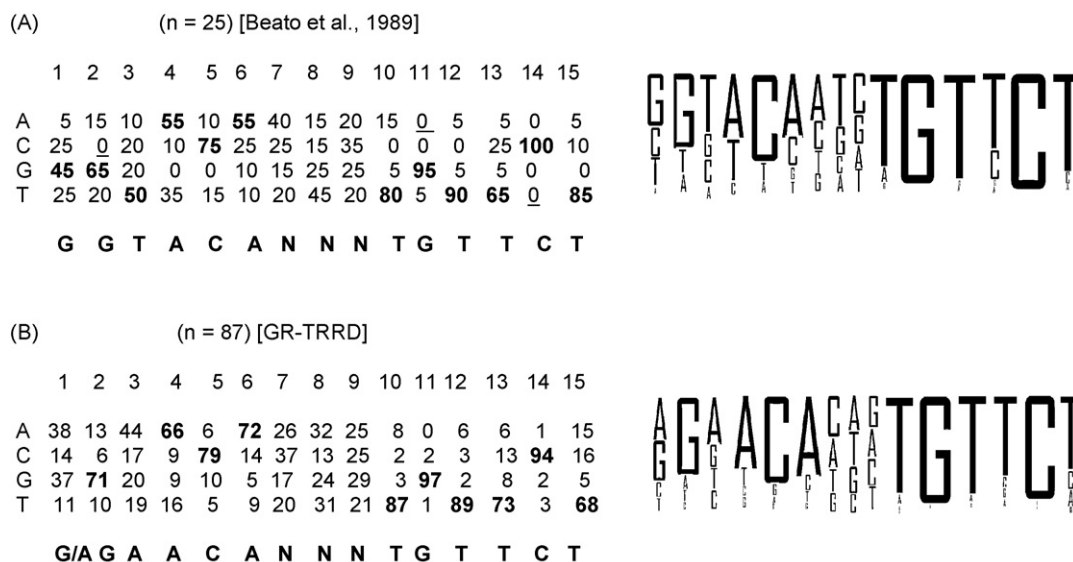|	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A	38	13	44	**66**	6	**72**	26	32	25	8	0	6	6	1	15
C	14	6	17	9	**79**	14	37	13	25	2	2	3	13	**94**	16
G	37	**71**	20	9	10	5	17	24	29	3	**97**	2	8	2	5
T	11	10	19	16	5	9	20	31	21	**87**	1	**89**	**73**	3	**68**

G/A G A A C A N N N T G T T C T

Fig. 1. Frequency matrices and consensuses obtained by analyzing (a) 25 GRbs [17] and (b) 87 GRbs from GR-TRRD. Nucleotide frequencies are shown as percent; graphic presentation the consensus sequence is to the left.

cleotide motifs – (1) TGTTCT in the case of GR and the receptors for mineralocorticoids (MR), progesterone (PR), and androgens (AR) and (2) TGACCT in the case of the rest receptors – are the basic elements of the nuclear receptor binding sites in DNA. In addition, the nuclear receptors are traditionally grouped according to the generally accepted patterns of their interaction with DNA and the structure of their binding sites. In particular, the steroid hormone receptors are usually described as the group of proteins interacting with DNA as homodimers, which recognize inverted repeats of the hexanucleotide TGTTCT (GR, MR, PR, and AR) or TGACCT (estrogen receptor) separated by a 3-bp spacer. The receptors for thyroid hormone (TR), vitamin D_3 (VDR), all-*trans* retinoic acid (RAR), retinoid X receptor (RXR), and manifold orphan receptors (HNF4, COUP, PPAR, CAR, PXR, LXR, etc.) are united into the group of proteins that interact as homo- or heterodimers with direct, inverted, or everted repeats of the motif TGACCT with a spacer varying from 0 to 9 bp in length. There is also the group of monomeric nuclear receptors (SF1, LRH1, ROR, ERR, etc.) containing in the binding site a single TGACCT motif preceded by 5′-flanking A/T rich sequence with a length of 3–6 bp [6–9].

However, the primary structure of many glucocorticoid receptor binding sites (GRbs) from various genes fails to comply with this pattern. There are GRbs containing only single copy of the hexanucleotide TGTTCT, and GR interacts with them as a monomer. The GRbs organized as a directly repeated hexanucleotide have been discovered. GR heterodimerization with other transcription factors has been reported. However, no systemic analysis of GRbs has been yet conducted. Here we describe the results of such analysis performed using the data on 160 GRbs complied in the GR-TRRD (Glucocorticoid-regulated genes TRRD; http://wwwmgs.bionet.nsc.ru/mgs/papers/merkulova/gluc/) section with the TRRD (Transcription Regulatory Regions Database; http://wwwmgs.bionet.nsc.ru/mgs/gnw/trrd/).

2. Structural variants of glucocorticoid receptor binding sites

GR was one of the first transcription factors purified to homogeneity as well as one of the first eukaryotic regulatory proteins shown to be capable of recognizing specific DNA regions [10–13]. In 1983–1984, the first GRbs consensus sequence – the hexanucleotide TGTTCT – was proposed. This sequence was discovered in DNA regions within chicken lysozyme gene promoter and the long terminal repeat of mouse mammary tumor virus (MMTV LTR), protected by GR from DNase I digestion (GR footprints) [14,15], and its variant, TGTTCC, in the GR footprint from promoter region of human metallothionein IIA gene [16]. Then the analysis of the data on 25 GRbs from various genes, available at that time, suggested the consensus GGTACAnnnTGTTCT (Fig. 1a) [17], which is an imperfect inverted repeat of the earlier discovered hexanucleotide with a spacer of 3 bp. The GRbs homologous to this consensus are called palindromic.

The volume of information about the arrangement of regulatory regions in the glucocorticoid-regulated genes and the mechanisms of glucocorticoid regulation have substantially increased over more than 20 years that passed since the discovery of the GR binding regions in DNA as well as the number of discovered GRbs. We are compiling this information in the specialized section of TRRD database, GR-TRRD [18].

2.1. GR-TRRD content: genes

Being a part of the TRRD database, GR-TRRD utilizes its system for recording the published experimental data [19–21]. The database entry is a gene. GR-TRRD compiles only the glucocorticoid-regulated genes for which the GR binding to DNA in their regulatory regions has been demonstrated. At present, GR-TRRD comprises descriptions of 86 glucocorticoid-regulated genes – 29 human, 32 rat, 15 mouse, 3 anthropoid primate, 2 chicken, 2 frog genes, 1 rabbit, 1 sheep, and 1 cow gene – and the LTR of two proviruses.

Of the genes compiled in GR-TRRD, 71 are up-regulated by glucocorticoids, 9 are down-regulated, and 6 can be both negatively and positively regulated by these hormones. Both LTRs provide a glucocorticoid induction. The fraction of genes down-regulated by glucocorticoids in GR-TRRD, 10%, is considerably lower than the estimates obtained in the microarray experiments on identification of glucocorticoid-regulated genes in various cell types, namely, 30–70% [22–28]. Thus, the selection of genes for GR-TRRD based on GR binding to DNA has resulted in an evident bias towards the genes up-regulated by glucocorticoids. This is another evidence confirming the accepted concept that the negative glucocorticoid regulation more frequently involves the

Table 1
The contents of palindromic and hexanucleotide GRbs in the genes up- and down-regulated by glucocorticoids.

	Up-regulated genes	Down-regulated genes	Combined regulation	Total
Palindromic GRbs	76	6	5	87
Hexanucleotide GRbs	46	17	1	64
Number of genes in group	65	8	4	77

mechanisms of GR–protein interaction rather than the GR–DNA binding [5,29–33].

2.2. GR-TRRD content: GRbs

Overall, GR-TRRD contains 174 GRbs. However, only the GRbs demonstrated experimentally to actually contact directly the receptor protein were used for analyzing the structural organization of the binding sites. The criterion for selecting the analyzed GRbs was the data on interaction between GR and the corresponding DNA region obtained by at least one of the following methods: (1) DNase I footprinting using purified GR or GRDBD (TRRD assay code 1.1.5.); (2) genomic footprinting under hormone treatment conditions (assay codes 1.5. and 6.5); (3) EMSA with purified GR or GRDBD (assay code 3.5.), extract from GR overproducer cells (assay code 3.5.1.), nuclear or cell extract and specific antibodies (assay code 3.6.); (4) methylation protection assay with purified GR (assay code 4.1); (5) methylation interference studies with purified GR (assay code 4.2); (6) ABCD assay (avidin–biotin complex DNA-binding assay) with purified GR (assay code 8). Finally, 160 GRbs from 77 genes were included into the sample (see Supplemental Table).

2.3. GRbs structural variants

Analysis of the nucleotide sequences of 160 sites forming the GRbs sample has demonstrated that only slightly more than half-sites (namely, 87) are palindromic. For these sites, the number of mismatches with the inverted repeat AGAACAnnnTGTTCT does not exceed six; moreover, each half-site displays no more than three mismatches. Characteristic of a large part of these GRbs is a high degree of homology to this consensus sequence. The number of mismatches does not exceed three for 52 of such sites, with no more than two mismatches for each half-site. However, 40% of the sites in GRbs sample (64 of 160) contain a single hexanucleotide TGTTCT (the number of mismatches varies from 0 to 2), whereas the 5′ flanking region of this hexanucleotide displays no more than two matches with the left part of the repeat. In addition, the matches in none of the 19 sites with two matches concurrently occur at the G at position 2 and the C at position 5, which are critical for GR binding [34]. Table 1 lists the rates of palindromic and hexanucleotide GRbs in the glucocorticoid-induced and -repressed genes. It is evident that the genes whose transcription is down-regulated by glucocorticoids much more frequently contain hexanucleotide sites than the palindromic variants. This fits well a rather old hypothesis that GR monomers are mainly involved in the mechanisms of DNA-mediated trans-repression [35]. However, a high rate of the hexanucleotide sites (over one third of the total number) in the glucocorticoid-inducible genes (Table 1) is an unexpected result.

In addition, the GRbs sample contains three sites organized as a direct repeat of the hexanucleotide TGTTCT (interacting with the heterodimer of glucocorticoid and mineralocorticoid receptors [36,37]); four GR-binding regions lacking any homology of this hexanucleotide; one site containing two overlapping palindromes (interacting with GR tetramer [38]); and one site where the palindrome and hexanucleotide overlap (interacting with GR trimer [39]).

2.4. Detailing of palindromic GRbs consensus

Expansion of the sample of GR binding sites and their grouping made it possible to determine the consensus of palindromic sites more precisely. Fig. 1b shows the frequency matrix and consensus, which we obtained based on the analysis of 87 GRbs sequences containing both halves of the inverted repeat. Comparison of the matrix and consensus with those obtained by Beato et al. [17] demonstrates that the increase in sample volume and exclusion of the half-sites elevated the conservation rate of ACA motif at positions 4–6 in the left part. On the other hand, the rate of G at position 1 somewhat decreased: A occurs at this position with the same frequency as G. Position 3 underwent radical changes, as it can contain any nucleotide instead of T, specified in Beato's consensus, with a certain preference of A. The conservation of the right part remained at approximately the same level as in the previous consensus with a certain decrease in the rate of T at the last position.

Two variants of palindromic nucleotide sequence are now used in searching for potential GRbs: more frequently, Beato's consensus GGTACAnnnTGTTCT [17] and less frequently, the perfect inverted repeat AGAACAnnnTGTTCT. Our results demonstrate that the variant AGAACAnnnTGTTCT describes the palindromic sites somewhat better than GGTACAnnnTGTTCT; however, the new consensus variant, G/AGnACAnnnTGTTCT, is more appropriate in searching for such sites.

2.5. Indispensability of 3-bp spacer and separation of TGTTCT and TGACCT motifs

An important result obtained by analyzing the overall sample of 160 GRbs nucleotide sequences is the discovered invariance of the 3-bp spacer between the halves of inverted repeat. Only one of all GRbs in the sample contains the spacer of another length (4 bp). Earlier studies of the palindromic GRbs from the MMTV LTR and −2.5-kb enhancer of rat tyrosine aminotransferase gene (TAT) have demonstrated that an artificial increase (to 4 bp) or decrease (to 2 bp) of the spacer leads to a drastic decrease in the affinity for GR and loss of the ability to provide glucocorticoid induction [40,41]. Our results not only fit well the experimental data obtained with these particular sites, but also suggest a general character of this property of the palindromic GRbs.

A considerable number of GRbs somewhat differ from the consensus sequence. GR belong to the small group of nuclear receptors (subfamily 3 group C, containing also MR, PR and AR [42]) with TGTTCT motif in their binding sites, whereas the other nuclear receptors interact with the motif TGACCT [6–9]. Correspondingly, it was most interesting to find out whether the observed deviations from the consensus led to formation of a "foreign" motif in any naturally occurring binding sites for his protein. The data shown in Fig. 1b demonstrate that the T → A substitution at position 12 (position 3 of the hexanucleotide TGTTCT) and T → C substitution at position 13 (position 4 of the hexanucleotide) occur in some sites. Similar substitutions occur in the corresponding positions of the inverted hexanucleotide copy (left part of the repeat) (Fig. 1b) as well as in the GRbs containing a single hexanucleotide (data not shown). However, these substitutions do not present simultaneously in any of GRbs from our sample, i.e., no hexanucleotide contained the dinucleotide AC at positions 3 and 4. Presumably, this suggests certain evolutionary mechanisms preserving separation of the sites for two groups of nuclear receptors.

3. Possible mechanisms underlying the function of hexanucleotide GRbs in glucocorticoid induction

It is known that GR binds to palindromic sites as a homodimer and to single hexanucleotides, as a monomer [43–45]. The affinity of GR dimer for palindromic sites is by one order of magnitude higher than the affinity of GR monomer for hexanucleotides [44,46–49]. It has been found that the GR binding to palindrome comprises the interaction of one receptor protein molecule (monomer) with the right part of repeat (TGTTCT, better half-site) followed by the binding of the second molecule to the worse half-site, thereby stabilizing the GR–DNA complex [41,44,50]. The mechanism of concerted GR interaction with the palindromic site involves an allosteric effect of the site on the conformation of receptor protein. In particular, it has been demonstrated that the binding of one receptor protein to the palindrome changes the conformation of its DNA-binding domain (DBD), namely, reorients its D loop, thereby creating a dimerization interface for the other GR monomeric subunit [51]. Mutations in DBD (*Ser459Ala*) and D loop (*Pro493Arg*) lead to a constitutive dimerization interface, inducing GR dimerization on a nonspecific DNA [52].

It has been demonstrated that the palindromic sites as a rule provide glucocorticoid induction of reporter genes upon linking a single copy to the heterologous promoter [48,53–57]. On the contrary, insertion of single hexanucleotide sites into such constructs fails to produce glucocorticoid inducibility [44,55,56–60]. However, analysis of our GRbs sample revealed a large number of hexanucleotide sites in the genes up-regulated by glucocorticoids (Table 1); moreover, a half of these sites were demonstrated to be directly involved in the glucocorticoid induction. Analysis of the data compiled in GR-TRRD suggests several mechanisms explaining how GR monomers and hexanucleotide half-sites act in up-regulation.

3.1. Interaction of GR monomers with other transcription factors (heterodimerization)

The interaction between GR monomer and the noncognate protein XGRAF, whose binding site is immediately adjacent to the GR binding site, hexanucleotide TGTTCC, located at positions −168/−162 in the promoter region of *Xenopus laevis* γ-fibrinogen subunit gene (TRRD accession no. A00734) is the most well-studied example of heterodimerization (Fig. 2). The authors named the united binding site for GR and XGRAF the glucocorticoid responsive unit (GRU) [61]. This GRU has been demonstrated to render

the foreign promoter glucocorticoid-inducible [62,63]. A direct protein–protein interaction between GR and XGRAF has also been shown as well as the fact that binding of any factor (GR or XGRAF) to GRU increases 30-fold the affinity of the partner for this DNA–protein complex as compared with the GRU free of proteins [62]. Thus, another protein with a neighboring binding site stabilizes the binding of GR monomer to the hexanucleotide site in a similar manner as the second GR monomer stabilizes binding of the first GR monomer to the classical palindromic site [41,44,50].

A similar interaction of GR monomer with another protein, Ets2, takes place in the rat CYP27 gene promoter (A01395). This region contains only one detected GR binding site (hexanucleotide at position −266/−261 bp) necessary for glucocorticoid induction. However, glucocorticoid induction also requires the binding site for Ets transcription factors at position −250/−244 (Fig. 2). A direct contact between GR and Ets2 has been demonstrated; it involves the DNA-binding domains of these proteins [64]. In the region neighboring the promoter of ovine β1-adrenergic receptor gene (A01873), GR monomer interacts with a member of Myc/Max family as well as with an unknown homeodomain protein, whose binding sites are immediately adjacent to the hexanucleotide. These sites (region −614/−574) and the proteins interacting with them constitute a functional ensemble that provides a glucocorticoid induction of the gene in question [65] (Fig. 2). In mouse α-amylase 2 gene (A00871), this functional ensemble is formed due to the interaction of GR monomer with PTF1 factor, whose binding sites are located at a distance of 100 bp from one another [66] (Fig. 2). It has been demonstrated that in such situations, the proteins bound to such sites contact with DNA loop formation [67].

3.2. GR multimerization in binding to clusters of hexanucleotide sites

A well-studied cluster containing three hexanucleotides is the region −120/−80 bp in MMTV LTR (A00045) (Fig. 3). As has been shown, the GR multimer of three or four monomers binds to this region. The affinity of this multimer for the three-hexanucleotide cluster is equal to the affinity of GR dimer for the palindromic GRE (−191/−167 bp in MMTV LTR) [49]. The contributions of the MMTV LTR region −120/−80 bp, containing the cluster, and palindromic GRbs (−191/−167 bp) to glucocorticoid induction are also equal [68]. In the case of multimerization, the GR molecules interact without involvement of D loop of the DNA-binding domain [69,70], which is necessary for the formation of GR homodimers
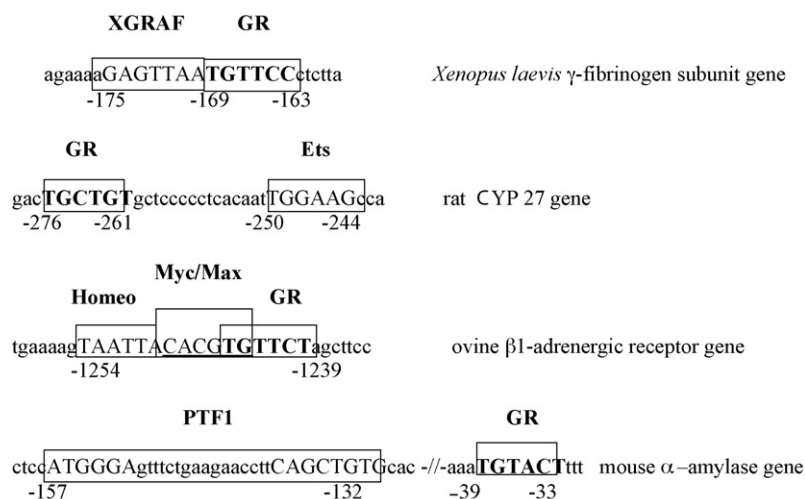

Fig. 2. Gene regions where GR heterodimerizes with other transcription factors. Rectangles denote transcription factor binding sites; hexanucleotide sites to which GR monomer binds are bold-faced; and figures show the distances to the transcription start site (translation stat site in the case of β1-adrenergic receptor).
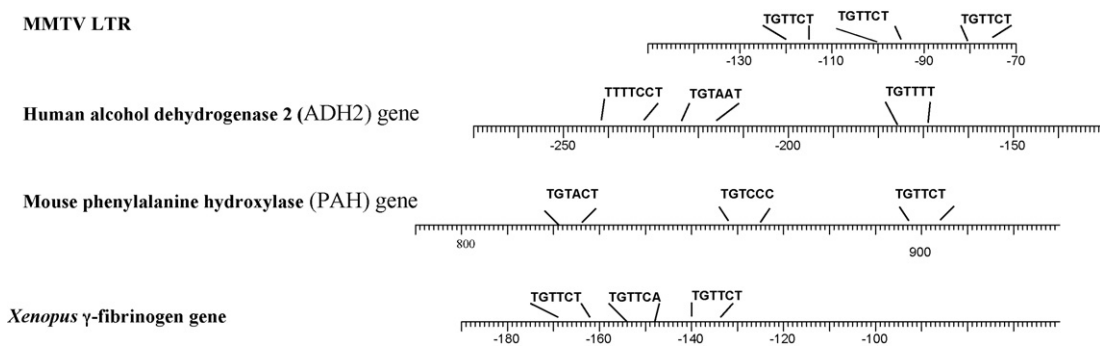
Fig. 3. Clusters of hexanucleotides in gene regulatory regions. Figures show the distances to the transcription start site (from the sequencing start in the case of mouse PAH gene enhancer). In human ADH2 and mouse RAH genes, hexanucleotides are located in the complementary DNA strand.

on palindromic sites. Another dimerization interface, located at the C-end of receptor molecule in the ligand-binding domain (LBD), is used in the multimerization [71]. In addition, certain data suggest that the N-end of receptor molecule can also be involved in the GR multimerization along with LBD [70]. The clusters of three hexanucleotides located within a region of 71–88 bp, which provide glucocorticoid induction, are also found in the genes of human alcohol dehydrogenase (A00379) and mouse phenylalanine hydroxylase (A00768) (Fig. 3). The promoter region of *Xenopus* γ-fibrinogen gene (A00734) also has a similar cluster, yet its first hexanucleotide is contained in the GRU where GR and XGRAF form heterodimer (Fig. 3). It has been shown that the GRU contribution to glucocorticoid inducibility amounts to 40%; two downstream hexanucleotide sites account for the rest [62]. On the one hand, such configuration of GR binding sites in γ-fibrinogen gene can lead to the formation of GR trimer where one subunit interacts with XGRAF. On the other hand, taking into account the latest data on a dynamic nature of GR interaction with promoters [72–74], it looks more likely that additional weak GR binding sites are necessary for increasing the local receptor concentration in the promoter region of γ-fibrinogen gene and the probability of its interaction with XGRAF.

3.3. Functioning of hexanucleotide sites as auxiliary elements for palindromic GRbs

In the gene regulatory regions, hexanucleotide sites frequently neighbor the classical GR binding sites organized as inverted repeats. In several cases, it has been demonstrated that the hexanucleotide sites are necessary for increasing (or even realizing) the glucocorticoid response. For example, the palindromic GR binding site (GREII, −2500 bp) in the glucocorticoid responsive enhancer (−2.5 kb) of rat TAT gene (A00026) neighbors two hexanucleotide sites (GREIII, −2450 bp, and GREI, −2600 bp). It has been shown that the enhancer fragment containing only GREII provides a 15–20-fold glucocorticoid induction of the reporter gene; the fragment containing only GREIII is inactive, and the fragment containing both sites provides a 30-fold glucocorticoid induction (the role of GREI in the glucocorticoid induction has not been studied) [55]. A hexanucleotide GRbs is present in the human thyrotropin releasing hormone receptor gene (TRHR) (A02464) at position −892/−887 bp and a palindromic site, at position −623/−609 bp. Both sites are necessary for glucocorticoid induction, as a mutational damage of any site causes a complete loss of inducibility [75]. The promoter region of rat hepatic aryl sulfotransferase (SULT1A1) (A02453) contains the hexanucleotide in an immediate vicinity of the palindromic GRbs but in the other strand. Removal of the hexanucleotide halves the level of glucocorticoid induction of this gene [76].

Similar variants of combinations of the palindromic and hexanucleotide GRbs are present in the LTR of murine Moloney sarcoma

virus (A00079), rat carbamoyl phosphate synthetase 1 (CPS1; A00757) and angiotensinogen (A00060) genes, and human constitutive androstane receptor (CAR) gene (A02491). Although in all these cases only the GR binding to the corresponding regions has been demonstrated and their role in glucocorticoid induction has not been studied, we can assume that the hexanucleotide half-sites in these genes also assist the functioning of palindromic GRbs.

The mechanism underlying the functioning of the half-sites located in the vicinity of palindromic sites in glucocorticoid induction is still vague. However, the data on dynamic exchange of GR and gene regulatory regions [72–76] suggest that weak hexanucleotides GRbs may serve as "traps" that increase the local GR concentration in the vicinity of strong palindromic sites thereby increasing the probability of its binding to them.

In turn, all the considered GRE variants can be elements of complex regulatory units boosting the glucocorticoid response, tissue specificity, and so on [43,77,78]. For example, the MMTV LTR region responsible for glucocorticoid induction (−210/−30 bp) contains, along with GRbs [14,49], one NF1 and two OTF1 binding sites [79,80], which are necessary for full hormone responsiveness. In addition to the already mentioned GRbs [55], the glucocorticoid responsive enhancer (−2.5 kb) of rat TAT gene contains also multiple HNF3 and C/EBP binding sites [81] as well as the biding sites for Ets family members [82]. The interaction of all these factors with GR is necessary for the liver-specific glucocorticoid induction of TAT gene. In this process, the leading role of HNF3 family members in determining the amplitude of glucocorticoid induction has been demonstrated [83]. The cluster of three hexanucleotide GRbs in the enhancer of mouse phenylalanine hydroxylase gene is adjacent to HNF1, C/EBP, and NF1 binding sites, which also provides the liver-specific glucocorticoid induction of this gene [84]. Such examples are numerous, and some of them are systematized in the review of Schoneveld et al. [78].

4. Conclusions

Numerous terms are now used for describing the mechanisms of DNA-dependent glucocorticoid regulation, namely, glucocorticoid receptor binding site (GRbs), glucocorticoid response element (GRE), glucocorticoid receptor recognition element, palindromic GRE, GRE half-site, half-GRE, composite GRE, positive GRE; negative GRE, tethering GRE, etc. However, the basic concepts – glucocorticoid receptor binding site (GRbs) and glucocorticoid responsive element (GRE) – are used as synonyms in the majority of the relevant papers. All the authors consider separately only the so-called tethering GRE, as these elements are the binding sites for other transcription factors rather than GRbs. GR interacts with them via a protein–protein manner and thus influences transcription of the genes containing such elements. On the other hand, not every site where GR specifically binds to DNA (GRbs) is a GRE.

The binding site for any transcription factor on DNA is a region of this molecule whose affinity for the protein in question is elevated because of a specific nucleotide sequence, which distinguishes it from the other DNA regions. For GR, this sequence is the hexanucleotide TGTTCT, which binds one receptor molecule. The equilibrium dissociation constant of such complex is 9.1×10^{-10} M, which is by two orders of magnitude ($\sim$400-fold) smaller than the dissociation constant for GR complex with a nonspecific DNA [44,49]. Thus, the hexanucleotide is actually the GRbs, which was reflected in the earliest consensus variant, TGTTCT [14–16]. However, the GR–hexanucleotide complex is insufficiently stable for this site to provide a glucocorticoid induction, i.e., to function as a regulatory element (GRE or more precisely, positive GRE) [44,55,58–60].

The positive GRE are formed involving hexanucleotide sites. Here, three main principles are used for increasing the stability of receptor–DNA complex. The most widespread pattern is duplication of the hexanucleotide to form an inverted repeat with a 3-bp spacer. This particular variant is known as the classical palindromic GRbs and reflected in the commonly accepted consensuses of this element, GGTACAnnnTGTTCT and AGAACAnnnTGTTCT. A GR homodimer is assembled at such sites, thereby increasing the stability of GR–DNA complexes by approximately one order of magnitude; correspondingly, such complexes acquire the ability to provide a glucocorticoid induction. Thus, two GRbs constitute the regulatory element, GRE.

However, the same results can be achieved by repeating the hexanucleotide GRbs in the same orientation more than twice. An example is the GRE from MMTV LTR, organized as a cluster of three hexanucleotides, where GR multimer is formed (Fig. 3). The stability of such complex is comparable with that of the classical complexes GR homodimer–palindromic site, and this complex is capable of providing glucocorticoid induction [49,68]. Presumably, this is not the unique case of forming a positive GRE through GR multimerization on the cluster of hexanucleotides. In particular, our sample of 77 glucocorticoid-regulated genes contains at least three more similar clusters. Assuming that the number of glucocorticoid-regulated genes in the mammalian genome exceeds one thousand, one can expect the discovery of several dozens of GRE organized according to this pattern.

Another way to increase the stability of the complex in question is GR heterodimerization with other transcription factors, when the binding of GR monomer to the hexanucleotide site is stabilized by another protein which has the binding site adjacent to the hexanucleotide. In these cases, the other factor actually plays the role of the second GR monomer, which stabilizes the binding of the first monomer to the palindromic site. An illustrative example is the heterodimerization of GR and XGRAF (Fig. 2) [62]. There are several other examples of confirmed interactions between the GR monomer bound to hexanucleotide and other transcription factors, which are necessary for glucocorticoid induction. As the regulatory regions of many glucocorticoid-regulated genes are yet insufficiently studied, we can assume that the GRE organized according to this pattern are much more numerous. First and foremost, such GRE can be found in a comprehensive study of the gene regions responsible for glucocorticoid induction with the already detected single hexanucleotides, necessary for its realization.

Thus, a large volume of our collection of experimentally detected GRbs allowed us to group them according to the type of conserved nucleotide sequence and to find new variants of positive GRE structural organization. The results of our study demonstrate that the generally accepted consensus of the GR binding sites – GGTACAnnnTGTTCT (AGAACAnnnTGTTCT) – actually describes the pattern of GRE structure that is most widespread in nature. However, there are other patterns of GRE organization, displayed by almost half of these elements.

Acknowledgments

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jsbmb.2009.02.003.

References

[1] K. Hierholzer, H. Buhler, Metabolism of cortical steroid hormones and their general mode of action, in: R. Greger, U. Windhorst (Eds.), Comprehensive human physiology, Springer-Verlag, Berlin, Heidelberg, 1996, pp. 79–93.

[2] N.Z. Lu, S.E. Wardell, K.L. Burnstein, D. Defranco, P.J. Fuller, V. Giguere, R.B. Hochberg, L. McKay, J.M. Renoir, N.L. Weigel, E.M. Wilson, D.P. McDonnell, J.A. Cidlowski, International union of pharmacology. LXV. The pharmacology and classification of the nuclear receptor superfamily: glucocorticoid, mineralocorticoid, progesterone, and androgen receptors, Pharmacol. Rev. 58 (2006) 782–797.

[3] H.M. Reichardt, G. Schutz, Glucocorticoid signalling-multiple variations of a common theme, Mol. Cell Endocrinol. 146 (1998) 1–6.

[4] R. Kumar, E.B. Thompson, Gene regulation by glucocorticoid receptor: structure: function relationship, J. Steroid Biochem. Mol. Biol. 94 (2005) 310–319.

[5] O. Kassel, P. Herrlich, Crosstalk between the glucocorticoid receptor and other transcription factors: molecular aspects, Mol. Cell Endocrinol. 275 (2007) 13–29.

[6] D.J. Mangelsdorf, C. Thummel, M. Beato, P. Herrlich, G. Schutz, K. Umesono, B. Blumberg, P. Kastner, M. Mark, P. Chambon, R.M. Evans, The nuclear receptor superfamily: the second decade, Cell 83 (1995) 835–839.

[7] S. Khorasanizadeh, F. Rastinejad, Nuclear-receptor interactions on DNA-response elements, Trends in Biochem. Sci. 26 (2001) 384–390.

[8] A.N. Smirnov, Nuclear receptors: nomenclature, ligands, mechanisms of their effects on gene expression, Biochemistry (Mosc.) 67 (2002) 957–977.

[9] A. Aranda, A. Pascual, Nuclear hormone receptors and gene expression, Physiol. Rev. 81 (2001) 1269–1304.

[10] O. Wrange, J. Carlstedt-Duke, J.-A. Gustaffson, Purification of the glucocorticoid receptor from rat liver cytosol, J. Biol. Chem. 254 (1979) 9284–9290.

[11] F. Payvar, O. Wrange, J. Carlstedt-Duke, S. Okret, J.A. Gustafsson, K.R. Yamamoto, Purified glucocorticoid receptors bind selectively in vitro to a cloned DNA fragment whose transcription is regulated by glucocorticoids in vivo, Proc. Natl. Acad. Sci. U.S.A. 78 (1981) 6628–6632.

[12] J.T. Kadonaga, Regulation of RNA polymerase II transcription by sequence-specific DNA binding factors, Cell 116 (2004) 247–257.

[13] J.A. Gustafsson, Steroids and scientist, Mol. Endocrinol. 19 (2005) 1412–1417.

[14] F. Payvar, D. De Franco, G.L. Firestone, B. Edgar, O. Wrange, S. Okret, J.A. Gustafsson, K.R. Yamamoto, Sequence-specific binding of glucocorticoid receptor to MMTV DNA at sites within and upstream of transcribed region, Cell 35 (1983) 381–392.

[15] R. Renkawitz, G. Schutz, D. von der Ahe, M. Beato, Sequence in the promoter region of the chicken lysozyme gene required for steroid regulation and receptor binding, Cell 37 (1984) 503–510.

[16] M. Karin, H. Haslinger, H. Holtgreve, R.I. Richards, P. Krauter, H.M. Westphal, M. Beato, Characterization of DNA sequences through which cadmium and glucocorticoid hormones induce human metallothionein IIA gene, Nature 308 (1984) 513–519.

[17] M. Beato, G. Chalepakis, M. Schauer, E.P. Slater, DNA regulatory elements for steroid hormones, J. Steroid Biochem. 32 (1989) 737–747.

[18] T.I. Merkulova, V.M. Merkulov, R.L. Mitina, Mechanisms of glucocorticoid regulation and regulatory regions of genes, controlled by glucocorticoids: description in the TRDD database, Mol. Biol. (Mosk) 31 (1997) 714–725.

[19] A.E. Kel', N.A. Kolchanov, O.V. Kel', A.G. Romashchenko, E.A. Anan'ko, E.V. Ignat'eva, T.I. Merkulova, O.A. Podkolodnaia, I.L. Stepanenko, A.V. Kochetov, F.A.F.A. Kolpakov, N.L. Podkolodnyĭ, A.A. Naumochkin, TRRD: a database of transcription regulatory regions in eukaryotic genes, Mol. Biol. (Mosk) 31 (1997) 626–636.

[20] N.A. Kolchanov, E.A. Ananko, O.A. Podkolodnaya, E.V. Ignatieva, I.L. Stepanenko, O.V. Kel-Margoulis, A.E. Kel, T.I. Merkulova, T.N. Goryachkovskaya, T.V. Busygina, F.A. Kolpakov, N.L. Podkolodny, A.N. Naumochkin, A.G. Romashchenko, Transcription Regulatory Regions Database (TRRD):its status in 1999, Nucleic Acids Res. 27 (1999) 303–306.

[21] N.A. Kolchanov, E.V. Ignatieva, E.A. Ananko, O.A. Podkolodnaya, I.L. Stepanenko, T.I. Merkulova, M.A. Pozdnyakov, N.L. Podkolodny, A.N. Naumochkin, A.G. Romashchenko, Transcription Regulatory Regions Database (TRRD): its status in 2002, Nucleic Acids Res. 30 (2002) 312–317.

[22] B.M. Agbemafle, T.J. Oesterreicher, C.A. Shaw, S.J. Henning, Immediate early genes of glucocorticoid action on the developing intestine, Am. J. Physiol. Gastrointest. Liver Physiol. 288 (2005) G897–G906.

[23] V. Gupta, A. Galante, P. Soteropoulos, S. Guo, B.J. Wagner, Global gene profiling reveals novel glucocorticoid induced changes in gene expression of human lens epithelial cells, Mol. Vis. 11 (2005) 1018–1040.

[24] S.L. Planey, M.T. Abrams, N.M. Robertson, G. Litwack, Role of apical caspases and glucocorticoid-regulated genes in glucocorticoid-induced apoptosis of pre-B leukemic cells, Cancer Res. 63 (2003) 172–178.

[25] J.-C. Wang, M.K. Derynck, D.F. Nonaka, D.B. Khondabakhsh, C. Haqq, K.R. Yamamoto, Chromatin immunoprecipitation (ChIP) scanning identified primary glucocorticoid receptor target genes, Proc. Natl. Acad. Sci. U.S.A. 101 (2004) 15603–15608.

[26] Z. Wang, M.H. Malone, H. He, K.S. McColl, C.W. Distelhorst, Microarray analysis uncovers the induction of the proapoptotic BH3-only protein Bim in multiple models of glucocorticoid-induced apoptosis, J. Biol. Chem. 278 (2003) 23861–72386.

[27] N.Z. Lu, J.A. Cidlowski, Translational regulatory mechanisms generate N-terminal glucocorticoid receptor isoforms with unique transcriptional target genes, Mol. Cell 18 (2005) 331–342.

[28] P. Phuc Le, J.R. Friedman, J. Schug, J.E. Brestelli, J.B. Parker, I.M. Bochkis, K.H. Kaestner, Glucocorticoid receptor-dependent gene regulatory networks, PLoS Genet. 1 (2005) e16.

[29] H.M. Reichardt, K.H. Kaestner, J. Tuckermann, O. Kretz, O. Wessely, R. Bock, P. Gass, W. Schmid, P. Herrlich, P. Angel, G. Schutz, DNA binding of the glucocorticoid receptor is not essential for survival, Cell 93 (1998) 531–541.

[30] M. Karin, New twists in gene regulation by glucocorticoid receptor: is DNA binding dispensable, Cell 93 (1998) 487–490.

[31] C. Stahn, M. Löwenberg, D.W. Hommes, F. Buttgereit, Molecular mechanisms of glucocorticoid action and selective glucocorticoid receptor agonists, Mol. Cell Endocrinol. 275 (2007) 71–78.

[32] A. Kleiman, J.P. Tuckermann, Glucocorticoid receptor action in beneficial and side effects of steroid therapy: lessons from conditional knockout mice, Mol. Cell Endocrinol. 275 (2007) 98–108.

[33] S. Bilodeau, S. Vallette-Kasic, Y. Gauthier, D. Figarella-Branger, T. Brue, F. Berthelet, A. Lacroix, D. Batista, C. Stratakis, J. Hanson, B. Meij, J. Drouin, Role of Brg1 and HDAC2 in GR trans-repression of the pituitary POMC gene and misexpression in Cushing disease, Genes Dev. 20 (2006) 2686–2871.

[34] C. Scheidereit, M. Beato, Contacts between receptor and DNA double helix within a glucocorticoid regulatory element of mouse mammary tumor virus, Proc. Natl. Acad. Sci. U.S.A. 81 (1984) 3029–3034.

[35] J.A. Lefstin, K.R. Yamamoto, Allosteric effects of DNA on transcriptional regulators, Nature 392 (1998) 885–888.

[36] X.M. Ou, J.M. Storring, N. Kushwaha, P.R. Albert, Heterodimerization of mineralocorticoid and glucocorticoid receptors at a novel negative response element of the 5-HT1A receptor gene, J. Biol. Chem. 276 (2001) 14299–14307.

[37] A. Derfoul, N.M. Robertson, J.B. Lingrel, D.J. Hall, G. Litwack, Regulation of the human Na/K-ATPase beta1 gene promoter by mineralocorticoid and glucocorticoid receptors, J. Biol. Chem. 273 (1998) 20702–20711.

[38] M. Garlatti, M. Daheshia, E.P. Slater, J. Bouguet, J. Hanoune, M. Beato, R. Barouki, A functional glucocorticoid responsive unit composed of two overlapping inactive receptor-binding sites: evidence for formation of receptor tetramer, Mol. Cell Biol. 14 (1994) 8007–8017.

[39] J. Drouin, Y.L. Sun, M. Chamberland, Y. Gauthier, A. De Lean, M. Nemer, T.J. Schmidt, Novel glucocorticoid receptor complex with DNA element of the hormone repressed POMC gene, EMBO J. 7 (1993) 3389–3395.

[40] G. Chalepakis, M. Schauer, X. Cao, M. Beato, Efficient binding of glucocorticoid receptor to its responsive element requires a dimer and DNA flanking sequences, DNA Cell Biol. 9 (1990) 355–368.

[41] K. Dahlman-Wright, A. Wright, J.A. Gustafsson, J. Carlstedt-Duke, Interaction of the glucocorticoid receptor DNA-binding domain with DNA as a dimer is mediated by a short segment of five amino acids, J. Biol. Chem. 226 (1991) 3107–3112.

[42] Nuclear Receptors Nomenclature Committee, A unified nomenclature system for the nuclear receptor superfamily, Cell 97 (1999) 161–163.

[43] M. Truss, M. Beato, Steroid hormone receptors: interaction with deoxyribonucleic acids and transcription factors, Endocr. Rev. 14 (1993) 459–478.

[44] J. Drouin, Y.L. Sun, S. Tremblay, P. Lavender, T.J. Schmidt, A. deLean, M. Nemer, Homodimer formation is rate-limiting for high affinity DNA binding by glucocorticoid receptor, Mol. Endocrinol. 6 (1992) 1299–1309.

[45] I. Segard-Maurel, K. Rajkowski, N. Jibard, C. Schweizer-Groyer, E.E. Bauleau, F. Cadepond, Glucocorticosteroid receptor dimerization investigated by analysis of receptor binding to glucocorticosteroid responsive elements using a monomer-dimer equilibrium model, Biochemisrty 35 (1996) 1634–1642.

[46] I. Alroy, L.P. Freedman, DNA binding analysis of glucocorticoid receptor specificity mutants, Nucleic Acids Res. 20 (1992) 1045–1052.

[47] T. Hard, K. Dahlman, J. Carlstedt-Duke, J.A. Gustafsson, R. Rigler, Cooperativity and specificity in the interactions between DNA and the glucocorticoid receptor DNA-binding domain, Biochemistry 29 (1992) 5358–5364.

[48] D.J. Rozansky, H. Wu, K. Tang, R.J. Parmer, D.T. O'Connor, Glucocorticoid activation of chromogranin A gene expression, J. Clin. Invest. 94 (1994) 2357–2368.

[49] T. Perlmann, P. Eriksson, O. Wrange, Quantitative analysis of glucocorticoid receptor-DNA interaction at the mouse mammary tumor virus glucocorticoid response element, J. Biol. Chem. 256 (1990) 17222–17229.

[50] S.Y. Tsai, J. Carlstedt-Duke, N. Weigel, K. Dahlman, J.A. Gustafsson, M.J. Tsai, B. O'Malley, Molecular interactions of steroid hormone receptor with its enhancer element: evidence of receptor dimer formation, Cell 55 (1988) 361–369.

[51] T. Stockner, H. Sterk, R. Kaptein, A.M. Bonvin, Molecular dynamics studies of molecular switch of glucocorticoid receptor, J. Mol. Biol. 328 (2003) 325–334.

[52] M.A.A. van Tilborg, J.A. Lefstin, M. Kruiscamp, J.M. Teuben, R. Boelens, K.R. Yamamoto, R. Kaptein, Mutations in the glucocorticoid receptor DNA-binding domain mimic an allosteric effect of DNA, J. Mol. Biol. 301 (2000) 947–958.

[53] U. Strahle, W. Schmid, G. Schutz, Sinergistic action of the glucocorticoid receptor with transcription factors, EMBO J. 7 (1988) 3389–3395.

[54] E.P. Slater, O. Rabenau, M. Karin, J.D. Baxter, M. Beato, Glucocorticoid receptor binding and activation of a heterologous promoter by the first intron of human growth hormone gene, Mol. Cell Biol. 5 (1985) 2984–2992.

[55] H.M. Jantzen, U. Strahle, B. Gloss, F. Stewart, W. Schmid, M. Boshart, M.,R. Miksicek, G. Schutz, Cooperativity of glucocorticoid response elements located far upstream of tyrosine aminotransferase gene, Cell 49 (1987) 29–38.

[56] C. Rhodes, Y. Yamada, Characterization of glucocorticoid responsive element and identification of an AT-rich element that regulate the link protein gene, Nucleic Acids Res. 23 (1995) 2305–2313.

[57] T. Hagerty, W.W. Morgan, N. Elango, R. Strong, Identification of a glucocorticoid-responsive element in the promoter region of the mouse tyrosine hydroxylase gene, J. Neurochem. 76 (2001) 825–834.

[58] S. Argentin, Y.L. Sun, I. Lihrmann, T.J. Schmidt, J. Drouin, M. Nemer, Distal cis-acting promoter sequences mediate glucocorticoid stimulation of cardiac atrial natriuretic factor gene transcription, J. Biol. Chem. 226 (1991) 23315–23322.

[59] R. Miksicek, A. Heber, W. Schmid, U. Danesch, G. Posseckert, M. Beato, G. Schutz, Glucocorticoid responsiveness of the transcriptional enhancer of moloney murine sarcoma virus, Cell 46 (1986) 283–290.

[60] B.T. Vander Kooi, H. Onuma, J.K. Oeser, C.A. Svitek, S.R. Allen, C.W. Vander Kooi, W.J. Chazin, R.M. O'Brien, The glucose-6-phosphatase catalytic subunit gene promoter contains both positive and negative glucocorticoid response elements, Mol. Endocrinol. 19 (2005) 3001–3022.

[61] B. Morin, C. Zhu, G.R. Woodcock, M. Li, R.N. Woodward, L.A. Nichols, L.J. Holland, The binding site for Xenopus glucocorticoid receptor accessory factor and a single adjacent half-GRE form an independent glucocorticoid response unit, Biochemistry 10 (2000) 12234–12242.

[62] B. Morin, G.R. Woodcock, L.A. Nichols, L.J. Holland, Heterodimerization between the glucocorticoid receptor and unrelated DNA-binding protein Xenopus glucocorticoid receptor accessory factor, Mol. Endocrinol. 15 (2001) 458–466.

[63] B. Morin, L.A. Nichols, L.J. Holland, Flanking sequence composition differentially affects the binding and functional characteristics of glucocorticoid receptor homo- and heterodimers, Biochemistry 45 (2006) 7299–7306.

[64] J. Mullick, H.K. Anandatheerthavarada, G. Amuthant, S.V. Bhagwat, G. Biswas, N.K. Bhat, V. Camasamudram, S.E.P. Reddy, V. Rao, N.G. Avadhani, Physical interaction and functional synergy between glucocorticoid receptor and Ets2 proteins for transcription activation of the rat cytochrome P-450c27 promoter, J. Biol. Chem. 276 (2001) 18007–18017.

[65] Y.T. Tseng, J.P. Stabila, T.T. Nguyen, B.G. McGonnigal, J.A. Waschek, J.F. Padbury, A novel glucocorticoid regulatory unit mediates the hormone resposiveness of the beta1-adrenergic receptor gene, Mol. Cell Endocrinol. 181 (2001) 165–178.

[66] E.P. Slater, H. Hesse, J.M. Muller, M. Beato, Glucocorticoid receptor binding site in the mouse alpha-amylase 2 gene mediates response to the hormone, Mol. Endocrinol. 7 (1993) 907–914.

[67] S. Semsey, K. Virnik, S. Adhya, A gamut of loops: meandering DNA, Trends Biochem. Sci. 30 (2005) 334–341.

[68] E. Buetti, B. Kuhnel, Distinct sequence elements involved in glucocorticoid regulation of mouse mammary tumor virus promoter identified by linker scanning mutagenesis, J. Mol. Biol. 190 (1986) 379–389.

[69] W. Liu, J. Wang, G. Yu, D. Pearce, Steroid receptor transcriptional synergy is potentiated by disruption of the DNA-binding domain interface, Mol. Endocrinol. 10 (1996) 1399–1406.

[70] M. Adams, O.C. Meijer, J. Wang, A. Bhargawa, D. Pearce, Homodimerization of glucocorticoid receptor is not essential for response element binding: activation of phenylethanolamine N-methyltransferase gene by dimerization-defective mutants, Mol. Endocrinol. 17 (2003) 2583–2592.

[71] R.K. Bledsoe, V.G. Montana, T.B. Stanley, C.J. Delves, C.J. Apolito, D.D. McKee, T.G. Consler, D.J. Parks, E.L. Stewart, T.M. Willson, M.H. Lambert, J.T. Moore, K.H. Pearce, H.E. Xu, Crystal structure of glucocorticoid receptor ligand binding domain reveals a novel mode of receptor dimerization and coactivator recognition, Cell 110 (2002) 93–105.

[72] D.A. Stavreva, W.G. Müller, G.L. Hager, C.L. Smith, J.G. McNally, Rapid glucocorticoid receptor exchange at a promoter is coupled to transcription and regulated by chaperones and proteasomes, Mol. Cell Biol. 24 (2004) 2682–2697.

[73] C. Elbi, D.A. Walker, G. Romero, W.P. Sullivan, D.O. Toft, G.L. Hager, D.B. DeFranco, Molecular chaperones function as steroid receptor nuclear mobility factors, Proc. Natl. Acad. Sci. U.S.A. 101 (2004) 2876–2881.

[74] S.H. Meijsing, C. Elbi, H.F. Luecke, G.L. Hager, K.R. Yamamoto, The ligand binding domain controls glucocorticoid receptor dynamics independent of ligand release, Mol. Cell Biol. 27 (2007) 2442–2451.

[75] P.I. Hovring, V. Matre, A.K. Fjeldheim, O.P. Loseth, K.M. Gautvik, Transcription of the human thyrotropin-releasing hormone receptor gene—analysis of basal promoter elements and glucocorticoids response elements, Biochem. Biophys. Res. Commun. 257 (1999) 829–834.

[76] Z. Duanmu, T.A. Kocarek, M. Runge-Morris, Transcriptional regulation of rat hepatic aryl sulfotransferase (SULT1A1) gene expression by glucocorticoids, Drug Metab. Dispos. 8 (2001) 1130–1135.

[77] P.C. Lucas, D.K. Granner, Hormone response domains in gene transcription, Annu. Rev. Biochem. 61 (1992) 1131–1173.

[78] O.J. Schoneveld, I.C. Gaemers, W.H. Lamers, Mechanisms of glucocorticoid signaling, Biochem. Biophys. Acta 1680 (2004) 114–128.

[79] M.G. Cordingley, A.T. Riegel, G.L. Hager, Steroid-dependent interaction of transcription factors with the inducible promoter of mouse mammary tumor virus in vivo, Cell 48 (1987) 261–270.

[80] U. Bruggemeier, M. Kalff, S. Franke, C. Scheidereit, M. Beato, Nuclear factor I acts as a transcription factor on the MMTV promoter but competes with steroid hormone receptors for DNA binding, Cell 64 (1991) 565–572.

[81] T. Grange, J. Roux, G. Rigaud, R. Pictet, Cell-type specific activity of two glucocorticoid responsive units of rat tyrosine aminotransferase gene is associated with multiple binding sites for C/EBP and a novel liver-specific factor, Nucleic Acids Res. 19 (1991) 131–139.

[82] M.L. Espinas, J. Roux, J. Ghysdal, R. Pictet, T. Grange, Participation of Ets transcriptional factors in glucocorticoid response of the rat tyrosine aminotransferase gene, Mol. Cell Biol. 14 (1994) 4116–4125.

[83] J. Roux, R. Pictet, T. Grange, Hepatocyte nuclear factor 3 determines the amplitude of the glucocorticoid response of the rat tyrosine aminotransferase gene, DNA Cell Biol. 14 (1995) 385–396.

[84] D.M. Faust, A.-M. Catherin, S. Barbaux, L. Belcadi, T. Imaizumi-Scherrer, M.C. Weiss, The activity of the highly inducible mouse phenylalanine hydroxylase gene promoter is dependent upon a tissue-specific, hormone-inducible enhancer, Mol. Cell Biol. 16 (1996) 3125–3137.