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Acetylation-mediated Epigenetic Regulation of Glucocorticoid Receptor Activity: Circadian Rhythm-associated Alterations of Glucocorticoid Actions in Target Tissues

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Abstract

Glucocorticoids influence organ functions through the glucocorticoid receptor, a protein acetylated and deacetylated by several histone acetyltransferases and deacetylases. We reported that the circadian rhythm-related transcription factor “Clock”, a key component of the biological CLOCK with inherent histone acetyltransferase activity, acetylates glucocorticoid receptor lysines within its hinge region -a “lysine cluster” containing a KXKK motif- and represses its transcriptional activity. This Clock-induced repression of the glucocorticoid receptor activity is inversely phased to the diurnally circulating glucocorticoids and may act as a local counter regulatory mechanism to the actions of these hormones. Importantly, uncoupling of the central CLOCK-regulated hypothalamic-pituitary-adrenal-axis and peripheral CLOCK-mediated alterations of glucocorticoid action, such as chronic stress and frequent trans-time zone travel or night-shift work, may cause functional hypercortisolism and contribute to various pathologies. Thus, acetylation-mediated epigenetic regulation of the glucocorticoid receptor may be essential for the maintenance of proper time-integrated glucocorticoid action, significantly influencing human well-being and longevity.

Keywords

acetylation; circadian rhythm; Clock; histone acetyltransferase (HAT); histone deacetylase (HDAC); hypothalamic-pituitary-adrenal (HPA) axis; Sirt1

1. Introduction

Mammalian organisms are influenced by unforeseen changes in the environment called “stressors”, and thus, have developed a highly sophisticated and conserved system, the Stress System, to help deal with them. This system is composed of the hypothalamic-

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pituitary-adrenal (HPA) axis and the locus caeruleus/norepinephrine-autonomic nervous systems (Chrousos, 1995; Miller and O'Callaghan, 2002; Sheridan, 2003; Chrousos, 2009; Chrousos, 2010). The HPA axis consists of the parvicellular corticotropin-releasing hormone (CRH)- and arginine vasopressin (AVP)-secreting neurons located in the hypothalamic paraventricular nucleus (PVN), the corticotrophs of the pituitary gland, and the adrenal gland cortices (Elenkov et al., 2000; Chrousos, 2009; Kudielka and Wust, 2010). The PVN neurons release CRH and AVP into the hypophyseal portal system located under the median eminence of the hypothalamus in response to signals from higher brain regulatory centers. Secreted CRH and AVP reach the pituitary gland and synergistically stimulate the secretion of adrenocorticotropic hormone (ACTH) (Bao et al., 2008; Chrousos, 2009; Aguilera, 2010). ACTH released into the systemic circulation finally stimulates production and secretion of glucocorticoids from the cortex of the adrenal glands (Chrousos, 1995). Secreted glucocorticoids in turn suppress higher regulatory centers, the PVN and the pituitary gland, forming a closed negative feedback loop that aims to reset the activated HPA axis and restore its homeostasis (Chrousos, 1995; Arafah, 2006; Chrousos, 2009; Aguilera, 2010).

The stress-responsive HPA axis is essential for survival in mammals and has strong and diverse actions on every aspect of their physiology (Kino and Chrousos, 2005; Aguilera, 2010; Chrousos, 2010). Indeed, its end-effector molecules, the glucocorticoids, are necessary for proper functioning of virtually all organs and tissues, including the central nervous system (CNS), and the respiratory, cardiovascular, immune, and musculoskeletal systems (Eskandari and Sternberg, 2002; Kino and Chrousos, 2005). Upon exposure to stress, glucocorticoids secreted into the systemic circulation in large amounts dramatically alter physiology, influencing behavior, shifting intermediary metabolism towards catabolism and modulating immune function (Chrousos, 1995; Chrousos, 2001; Kino and Chrousos, 2005; Kyrou and Tsigos, 2009).

The activity of the Stress System is diurnally linked to the rotation of the planet and appropriately connected with the daily activity/rest of the organism and circulating glucocorticoid levels, ie. cortisol in humans and corticosterone in rodents, are under the strong circadian influence of the suprachiasmatic nucleus (SCN) of the hypothalamus (Chrousos, 1995; Nader et al., 2010). In humans, the cortisol diurnal zenith is reached in the early morning and the nadir at midnight, with the purpose of helping adjust the body’s activities to the regular periodicity of day/night changes. The time-integrated daily secretion of glucocorticoids is tightly regulated; indeed, the circadian, negative feedback and stress-related activities of the HPA axis are integrated “rheostatically” by higher brain centers (Chrousos, 1995; Nader et al., 2010).

The overall sensitivity of tissues to glucocorticoids, on the other hand, is also regulated by various physiologic and pathologic processes (Kino et al., 2003; Chrousos and Kino, 2005). For example, glucocorticoid action in cells is specifically adjusted during the different phases of the cell cycle (Cidlowski and Michaels, 1977; Abel et al., 2002), while adrenomedullary cells exposed to very high concentrations of glucocorticoids directly diffusing from the adjacent adrenal cortex, are resistant to these hormones in a gene-specific fashion (Wurtman, 2002; Ehrhart-Bornstein and Bornstein, 2008). In addition, several autoimmune/allergic/inflammatory disorders, the metabolic syndrome, septic conditions and even infection with the human immunodeficiency virus type-1, have been associated with alterations in the responsiveness of specific organs and tissues to glucocorticoids (Kino et al., 2003; Webster et al., 2004; Chrousos and Kino, 2005; Chrousos and Kino, 2007). Underlying mechanisms(s) for the alterations of local glucocorticoid actions, however, have not been fully elucidated as yet.

2. Glucocorticoid Receptor (GR) and Regulation of its Activities

The diverse actions of glucocorticoids at their target tissues are mediated by a single intracellular protein molecule the glucocorticoid receptor (GR) (Chrousos, 1995; Nicolaides et al., 2010) (Figure 1). This receptor, also known as the “nuclear receptor superfamily 3, group C, member 1 (NR3C1)”, is expressed virtually in all organs and tissues of the human body, and belongs to the steroid/sterol/thyroid/retinoid/orphan nuclear receptor superfamily, which consists of 48 members in humans (O'Malley, 1990; Kino et al., 2003; Chrousos and Kino, 2005). The human GR gene, located in the short arm of chromosome 5 (5q31.3), is composed of 9 exons, and encodes two protein molecules GRα and GRβ through alternative use of specific exons 9α and 9β (Kino et al., 2009; van der Vaart and Schaaf, 2009) (Figure 1). GRα is the ubiquitously expressed classic receptor that binds to and mediates most of the known actions of glucocorticoids, while GRβ , although also expressed widely, does not bind glucocorticoids and its physiologic actions have not been fully elucidated as yet (Kino et al., 2009). It recently became evident that the GRα variant mRNA is translated from at least 8 initiation sites into multiple amino terminal GRα isoforms, termed A through D (A, B, C1-C3 and D1-D3), with distinct specific transcriptional activities on glucocorticoid-responsive genes (Lu and Cidlowski, 2005).

The human GRα consists of 777 amino acids and has 3 major distinct functional domains, the N-terminal or immunogenic (NTD), the DNA-binding (DBD) and the ligand-binding (LBD) domains (Hollenberg et al., 1985; Kino and Chrousos, 2004) (Figure 1). GRα has also a hinge region (HD), located between the DBD and LBD and spanning amino acids 481 to 520 (Nader et al., 2009). GRα is located primarily in the cytoplasm in the absence of glucocorticoid ligand, as part of hetero-oligomeric complexes containing heat shock proteins (HSPs) 90, 70, 50, 20 and, possibly, other proteins as well (Pratt, 1993; Hager, 2002; Revollo and Cidlowski, 2009). After binding to its agonist ligand, GRα undergoes conformational changes, dissociates from the heat shock proteins, homo- or hetero-dimerizes, and translocates as a dimer and/or monomer into the nucleus through the nuclear pore, via an active ATP-dependent process mediated by its nuclear localization signals (NL)-1 and -2 (Savory et al., 1999). NL-1 is located in the junction of DBD and the hinge region, while NL-2 spans the entire LBD (Savory et al., 1999) (Figure 1).

Inside the nucleus, the ligand-activated GRα directly interacts as a homo- or hetero-dimer with specific DNA sequences, the glucocorticoid response elements (GREs), in the promoter regions of target genes, (Beato et al., 1989; Kino et al., 2003; Chrousos and Kino, 2005; So et al., 2007). GR contains two transactivation domains, activation function (AF)-1 and -2, located at its NTD and LBD, respectively (Figure 1), through which it interacts with many proteins and protein complexes, such as the nuclear receptor coactivator [p160, p300/CREB-binding protein (CBP) and p300/CBP-associated factor (p/CAF)] complexes and the SWI/SNF and vitamin D receptor-interacting protein/thyroid hormone receptor-associated protein (DRIP/TRAP) chromatin-remodeling complexes, eventually influencing the activity of the RNA polymerase II and its ancillary factors, modulating the transcription rates of glucocorticoid-responsive genes (McKenna and O'Malley, 2002; Kino and Chrousos, 2004; Chrousos and Kino, 2005; Rosenfeld et al., 2006).

In addition to transactivation or transrepression of the glucocorticoid-responsive genes explained above, GRα mutually modulates other signal transduction cascades through protein-protein interaction with specific transcription factors, by influencing the ability of these factors to stimulate or inhibit the transcription rates of their respective target genes (Barnes, 1998; Chrousos and Kino, 2005; Kino and Chrousos, 2005). This activity may be more important than the GRE-mediated one, granted that mice harboring a mutant GRα, which is active in terms of protein-protein interactions but inactive in terms of dimerization

and transactivation via DNA GREs, survive and procreate, in contrast to mice with a deletion of the entire GR gene that die immediately after birth from severe respiratory distress syndrome (Cole et al., 1995; Reichardt et al., 1998).

3. Acetylation of GR

In addition to co-regulators and other transcription factors that modulate GR-induced transcriptional activity, several distinct signaling pathways influence the transcriptional activity of the GR via post-translational modifications of the receptor protein (Chrousos and Kino, 2005). These include methylation, nitrosylation, sumoylation and ubiquitination, and phosphorylation, the last of which has been studied best. Indeed, several kinases, such as the cell-cycle-related kinases, mitogen-activated kinases (MAPKs) and the glycogen synthase kinases, phosphorylate specific serine or threonine residues of the GR, while energy sensing AMP-activated protein kinase indirectly phosphorylates GR through activation of p38 MAPK (Rogatsky et al., 1998; Itoh et al., 2002; Wang and Garabedian, 2003; Ismaili and Garabedian, 2004; Szatmary et al., 2004; Miller et al., 2005; Kino, 2007; Kino et al., 2007; Kino et al., 2010; Nader et al., 2010).

GR is also acetylated and this epigenetic modulation of the GR has important regulatory roles on the biologic actions of glucocorticoids in target tissues. Indeed, acetylation is a general epigenetic modulation that controls activity of various cytoplasmic as well as nuclear proteins (Minucci and Pelicci, 2006). Further, acetylation of nuclear proteins, including histones, plays important roles in the regulation of gene expression and subsequent alteration of biologic activities of organisms. For example, acetylation of the N-terminal tail of chromatin-associated histones by HAT coactivators is essential for initiating transcription by exposing the promoter DNA to transcription factors, other chromatin-modifying molecules and the RNA polymerase II complex (An, 2007). HAT coactivators also acetylate various other molecules, such as the coactivators themselves, other transcription intermediate components, several transcription factors, including nuclear receptors and chaperone molecules like HSP90 (Kovacs et al., 2005; Murphy et al., 2005; Minucci and Pelicci, 2006).

The human GR was first shown to be acetylated at lysines 494 and 495 located in its hinge region, while it was deacetylated by histone deacetylase 2 (HDAC2) (Ito et al., 2006) (Figure 2A). The acetylation of GR was demonstrated by employing an anti-acetylated lysine-specific antibody and the GR mutants defective in specific acetylation sites, but was not confirmed by methods directly detecting acetylated residues of the GR protein, such as the mass-spectrometry. Deacetylation of GR by this HDAC was required for efficient transrepression of nuclear factor of κ B (NF-κ B)-induced transcriptional activity by the GR (Ito et al., 2006). These findings indicate that acetylation of the GR at these lysine residues attenuates the repressive effect of this nuclear receptor on NF-κ B. These lysine residues of the GR are in the a common acetylation motif KXKK, where K is lysine and X is any amino acid (Figure 2A). This motif is shared by other steroid hormone receptors, the mineralocorticoid (MR), androgen (AR) and progesterone (PR) receptors, but not with the estrogen receptors (ER) α and β (Faus and Haendler, 2006), suggesting that acetylation is a general and conserved regulatory mechanism for several steroid hormone receptors. Indeed, the human androgen receptor is acetylated at lysines 632 and 633 located in the KXKK motif, while p300, p/CAF and the Tat-interacting protein 60 (Tip60) HAT coactivators are responsible for the acetylation of these residues (Fu et al., 2000; Gaughan et al., 2002). PR is also acetylated at lysines located in the KXKK motif (Gaviglio et al., 2010), whereas acetylation of the MR has not been shown as yet. Interestingly, the human ERα is acetylated by p300 at lysines 266, 268, 302 and 303, and this posttranslational modification increases binding to estrogen response elements (Faus and Haendler, 2006; Kim et al., 2006).

Acetylation of AR can be inhibited by addition of the class I/II HDAC inhibitor tricostatin-A (TSA), while p300-mediated acetylation of ERα is inhibited by TSA and the class III HDAC inhibitor nicotinamide, suggesting that AR and ERα are deacetylated by these HDACs, including HDAC1/2 and the class III HDAC Sirt1 (Fu et al., 2000; Kim et al., 2006). Recently, AR was shown to interact directly with Sirt1, and this HDAC efficiently deacetylated this receptor (Fu et al., 2006).

Temperature-activating factor (TAF)-Iβ and pp32, which are components of the inhibitor of histone acetyltransferases (INHAT), together with the other component TAF-Iα, also antagonize p300-mediated acetylation of ERα (Seo et al., 2001; Loven et al., 2004). Since GR can directly interact with p300 and TAF-Iβ , and is sensitive to TSA and Sirt1 (Ichijo et al., 2005; Amat et al., 2007; Ichijo et al., 2008), it is likely that GR is also acetylated by HAT coactivators, including p300, and deacetylated by class I/II HDACs and/or Sirt1 (Figure 2A). Acetylation of GR by HATs might also be influenced further by the INHAT components.

4. CLOCK-mediated Acetylation of GR: Implications to Circadian Rhythm-mediated Regulation of Glucocorticoid Action in Target Tissues

We recently found that the Clock transcription factor acetylated GR at its multiple lysine cluster in the hinge region, which includes lysines 494 and 495 located in the KXKK motif, and repressed GR-induced transcription of several glucocorticoid-responsive genes, by employing several GR mutants defective in acetylation sites and the anti-acetylated lysine-specific antibody (Nader et al., 2009). Clock appears to acetylate GR in the nucleus, after this receptor has bound glucocorticoids in the cytoplasm and has translocated into the nucleus. Clock, the “circadian locomotor output cycle kaput”, and its heterodimer partner “brain-muscle-arnt-like protein 1” (Bmal1) belong to the basic helix-loop-helix (bHLH)-PER-ARNT-SIM (PAS) superfamily of transcription factors, and play an essential role in the generation of the circadian oscillation rhythm of the CLOCK system that functions as an internal circadian time keeper (Takahashi et al., 2008). The Clock/Bmal1 heterodimer binds the E-box response elements located in the promoter region and stimulates the transcription of other essential clock genes, such as *Periods* (*Per1*, *Per2* and *Per3*) and *Cryptochromes* (*Cry1* and *Cry2*). Accumulated proteins Pers and Crys then form a complex with caseine kinase 1ε and δ, are phosphorylated, translocate into the nucleus and repress the transcriptional activity of the Clock/Bmal1 heterodimer by inhibiting its binding to the E-box response elements located in their own promoters, ultimately forming a negative feedback transcriptional loop that maintains an oscillation of their gene expression approximately every 24 hours (Kiyohara et al., 2006; Kondratov et al., 2006; Takahashi et al., 2008; Nader et al., 2010). In addition to the regulation of this principal transcriptional loop, Clock/Bmal1 stimulates expression of other CLOCK-related proteins, such as Rev-erbα, retinoic acid receptor-related orphan receptor α (RORα), Dec1, Dec2 and albumin gene D site-binding protein (Dbp), which form an auxiliary loop that stabilizes the main regulatory loop composed of Clock/Bmal1, Pers and Crys. Importantly, the transcription factors of the main and auxiliary loops control numerous “downstream” clock-responsive genes and influence a variety of biological activities (Ko and Takahashi, 2006; Ripperger and Schibler, 2006; Takahashi et al., 2008; Nader et al., 2010). The CLOCK transcription factor system located in the SCN of the brain hypothalamus, acts as the “master” oscillator and generator of the body’s circadian rhythm under the strong influence of the light/dark input from the eyes (Takahashi et al., 2008). The peripheral CLOCK system distributed in all organs and tissues, including the CNS outside the SCN, acts generally as a “slave” CLOCK under the regulation of the central SCN CLOCK by as yet unknown mechanisms;

both neuronal and humoral connections have been implicated (Takahashi et al., 2008; Nader et al., 2010).

Clock physically interacts with the GR LBD at its nuclear receptor-interacting domain (NRID) located in its middle portion, and acetylates human GR at amino acid 480, 492, 494 and 495 (Nader et al., 2009; Nader et al., 2010) (Figure 2A). Acetylation of GR attenuates binding of the receptor to GREs, and hence, represses GR-induced transactivation of GRE-driven promoters (Nader et al., 2009) (Figure 2B). The lysine residues acetylated by Clock are positioned in the C-terminal extension (CTE) located in the N-terminal portion of GR NTD, which is known to play a role in DNA recognition by steroid hormone receptors (Melvin et al., 2004). Thus, it is likely that acetylation of these residues reduces binding of GR to GREs in part by altering the action of CTE. The portion of the hinge region acetylated by Clock overlaps with NL-1 that spans the C-terminus of DBD to the hinge region and plays an essential role in the cytoplasmic to nuclear translocation of the receptor (Savory et al., 1999; Nader et al., 2009). Therefore, it is also possible that acetylation of GR alters nuclear translocation of this receptor. In addition to CTE and NL-1, the part having the KXKK acetylation motif in the AR hinge region suppresses the N/C interaction, which plays an important role in AR- and MR-induced transcriptional activity (Thompson et al., 2001; He et al., 2002; Rogerson and Fuller, 2003; Pippal et al., 2009). This portion of AR also has suppressive effect on p160 coactivator-mediated potentiation of AR transcriptional activity (Haelens et al., 2007). Thus, it appears that Clock-mediated acetylation may reduce GR transcriptional activity via multiple different modes of actions.

Phosphorylation of the N-terminal tail (serine residues) of histone H3 is a prerequisite for acetylation of the lysine residues of this molecule, indicating a functional link between phosphorylation and acetylation on the same target molecule (Cheung et al., 2000; Thomson et al., 2001; Clayton and Mahadevan, 2003). This concerted action of two major chemical modifications, which may represent molecular inputs from various extracellular and intracellular pathways, has been called “phosphoacetylation”. As the GR is sensitive to both phosphorylation and acetylation, it is possible that the former influences Clock-mediated acetylation and hence GR activity. This may provide another level of interaction between biological pathways that activate GR-targeting kinases, such as cell cycle-regulating kinases, p38 MAPK and AMPK, and the CLOCK-mediated circadian system, ultimately influencing the activity of glucocorticoid-responsive genes at target tissues in a complex fashion.

Circulating glucocorticoids fluctuate naturally in a circadian fashion, reaching their zenith in the early morning and their nadir in the late evening in diurnal animals, including humans (Chrousos, 1995; Chrousos, 2001). The light-activated central master CLOCK located in the SCN orchestrates this daily rhythmic release of glucocorticoids by influencing the central activity of the HPA axis through efferent connections from the SCN to the CRH/AVP-containing neurons of the PVN (Ishida et al., 2005; Kalsbeek et al., 2006; Bao et al., 2008). This central CLOCK-mediated diurnal regulation of circulating glucocorticoids is extremely important for adjusting the body’s daily activities. In addition to this central regulation of the HPA axis for glucocorticoid secretion by the adrenal glands, the central CLOCK system modulates glucocorticoid release from the adrenal cortex by altering its sensitivity to ACTH neurally through SCN-mediated activation of the autonomic nervous system (Ishida et al., 2005; Kalsbeek et al., 2006; Oster et al., 2006; Ulrich-Lai et al., 2006) (Figure 3). Since the circadian rhythm of the peripheral CLOCK system is synchronized to that of the central CLOCK and acetylation of GR by the peripheral CLOCK represses GR-induced transcriptional activity, acetylation-mediated regulation of GR transcriptional activity by Clock appears to function as a local counter regulatory mechanism to diurnally oscillating circulating glucocorticoids (Nader et al., 2010) (Figure 3).

Dysregulation as well as uncoupling of the cortisol circadian rhythm under the influence of the SCN CLOCK system, and acetylation-mediated circadian changes in local tissue sensitivity to glucocorticoids by the peripheral CLOCK, may lead to functional hypercortisolism in target tissues, which could be associated with development of pathologic conditions (Nader et al., 2010). For example, rotating shift workers, whose circadian system is repetitively reset by night-time activity/day-time sleep, and persons exposed to frequent jet lag due to frequent travels over time zones, are at an increased risk for cardiometabolic disease and stroke, as well as early mortality that might result from such time-integrated increased target tissue exposure to glucocorticoids (Ekstrand et al., 1996; Davidson et al., 2006; Scheer et al., 2009; Harrington). In experimental jet lag in mice, the HPA axis plays a critical role in adjusting the behavioral activity of these animals against let lag, while the peripheral CLOCK in the adrenal glands is important for resetting diurnal changes of circulating corticosterone secreted from these organs (Kiessling et al., 2010). Accordingly, chemical blocking of glucocorticoid secretion from the adrenal glands could either accelerate or prolong jet lag, depending on the timing of treatment (Kiessling et al., 2010). Thus, the aceylation-mediated negative regulation of GR transcriptional activity by the peripheral circadian CLOCK systems, including that of the adrenal glands, together with circadian regulation of CRH/AVP/ACTH/circulating glucocorticoids by the SCN, appear to play significant roles in physical as well as mental activities of humans, and dysregulation in any of these components could lead to development of various behavioral and/or somatic disorders.

5. Concluding Remarks

Acetylation of the GR appears to be fundamental for the regulation of its transcriptional activity, as the KXKK motif is conserved phylogenetically in most of the steroid hormone receptors including the GRs of all vertebrates after lampreys, including mammals, in comparison to the regulation via phosphorylation of serine residues by the serine/threonine kinases, based on the evidence that residues susceptible to phosphorylation are conserved only in mammals. In agreement with these observations, circadian rhythm transcription factor Clock, which is functional in the adaptive response to day/night changes that is essential for survival of virtually all organisms living on earth, acetylates GR at several lysines, including those in the KXKK acetylation motif located in the hinge region, and regulates GR-induced transcriptional activity. Thus, GR acetylation, including that by the CLOCK circadian system, play several important roles in the regulation of glucocorticoid action in target tissues. Consequently, disruption in the coupling between the HPA axis and circadian GR acetylation or dysregulation in GR acetylation may lead to development of various pathologic conditions, such as components of the metabolic syndrome and cardiovascular disorders, which strongly influence well-being and longevity in humans. Sirt1, a class III HDAC and one of the 7 human sirtuins, deacetylates several nuclear receptors and influences longevity; this HDAC might also deacetylate the GR, counteracting Clock-mediated regulation by decreasing GR-induced transcriptional activity (Blander and Guarente, 2004; Fu et al., 2006; Amat et al., 2007; Li et al., 2007). We suggest that the balance between acetylation and deacetylation of the GR promoted by Clock and, possibly, Sirt1, respectively, might be critical for the maintenance of proper biologic functioning of the GR, ultimately influencing well-being and longevity. This potentially important regulation of GR actions and its physiologic and pathophysiologic significance in humans begs for further studies.

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Abbreviation list

ACTH	Adrenocorticotropic hormone
Dbp	Albumin gene D site-binding protein
AMPK	AMP-activated protein kinase
AR	Androgen receptor
AVP	Arginine vasopressin
bHLH	Basic helix-loop-helix
Bmal1	Brain-muscle-arnt-like protein 1
CNS	Central nervous system
Clock	Circadian locomotor output cycle kaput
CRH	Corticotropin-releasing hormone
Cry	Cryptochrome
CTE	C-terminal extension
DBD	DNA-binding domain
ER	Estrogen receptor
GR	Glucocorticoid receptor
GRE	Glucocorticoid response element
HSP	Heat shock protein
HAT	Histone acetyltransferase
HDAC	Histone deacetylase
HPA axis	Hypothalamic-pituitary-adrenal axis
INHAT	Inhibitor of histone acetyltransferases
LBD	Ligand-binding domain
MR	Mineralocorticoid receptor
MAPK	Mitogen-activated kinase
NTD	N-terminal or immunogenic domain
NF-κ B	Nuclear factor of κ B
NL	Nuclear localization signal
NRID	Nuclear receptor-interacting domain
NR3C1	Nuclear receptor superfamily 3, group C, member 1
p/CAF	p300/CBP-associated factor
CBP	p300/CREB-binding protein

PVN	Paraventricular nucleus
PAS	PER-ARNT-SIM
Per	Period
PR	Progesterone receptor
RORα	Retinoic acid receptor-related orphan receptor α
SCN	Suprachiasmatic nucleus
Tip60	Tat-interacting protein 60
TAF-Iβ	Temperature-activating factor-Iβ
TSA	Tricostatin-A
DRIP/TRAP	Vitamin D receptor-interacting protein/thyroid hormone receptor-associated protein

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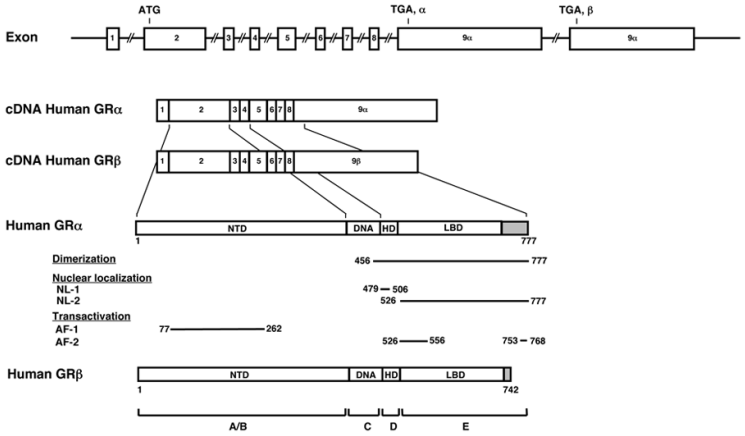

Figure 1. Genomic and complementary DNA, and protein structures of the human GR, functional domains distribution of GR*α*, and the GR isoforms produced through alternative splicing

The human GR gene consists of 10 exons. Exon 1 is untranslated region, exon 2 codes for the immunogenic domain (A/B), exon 3 and 4 for the DNA-binding domain (C), and exons 5-9 for the hinge region (D) and the ligand-binding domain (E). GR does not contain an F region, in contrast to the other steroid hormone receptors. The human GR gene contains two terminal exons 9 (exon 9α and 9β) alternatively spliced to produce the classic GRα and the nonligand-binding GRβ . C-terminal gray colored domains in GRα and GRβ show their specific portions. Locations of several functional domains are also indicated.

AF-1 and -2: activation function 1 and 2; DBD; DNA-binding domain; HD: hinge region; LBD: Ligand-binding domain; NTD: N-terminal region, NL1 and 2: Nuclear translocation signal 1 and 2.

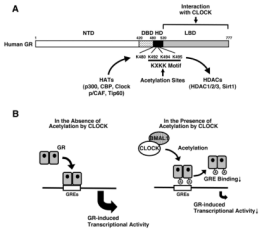

Figure 2. Acetylation sites of the GR and regulation of GR transcriptional activity by Clock

A: Multiple acetylation sites in the human GR and possible acetylases and deacetylases for the GR.

The human GR has 4 acetylation sites in its hinge region; lysines, 480, 492, 494 and 495. These lysine residues may be acetylated by several HATs, such as p300, CBP, Clock, p/CAF and Tip60, while they are several potential deacetylates including class I to III HDACs, such as HDCA1, 2, 3 and Sirt1. Clock physically interacts with GR LBD through the domain enclosed in its C-terminal part and acetylates GR at all lysine residues located in a lysine cluster of the hinge region.

Modified from (Nader et al., 2009).

B: A heuristic model of the physiologic implications of this study.

Clock/Bmal1 acetylates GR via its intrinsic HAT activity through physical interaction with GR LBD, reduces affinity of GR to its cognate DNA GREs and ultimately suppresses GR-induced transcriptional activity.

Modified from (Nader et al., 2009).

A: acetylation, Bmal1: brain-muscle-arnt-like protein 1, CBP: CRE-binding protein-binding protein, Clock: circadian locomotor output cycle kaput, DBD: DNA-binding domain, GR: glucocorticoid receptor, GRE: glucocorticoid response element, HAT: histone acetyltransferase, HDAC: histone deacetylase, HD: hinge region, LBD: ligand-binding domain, NTD: N-terminal domain, p/CAF: p300/CBP-associated factor, Tip60: Tat-interacting protein 60

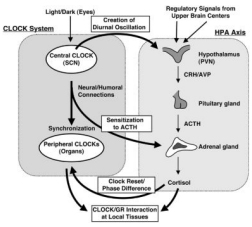

Figure 3. The circadian CLOCK system and the HPA axis influence each other's activity at multiple levels

The central CLOCK under the regulation of the light input controls and overrides the HPA axis and produces regular diurnal secretion of glucocorticoid hormones from the adrenal glands, while the peripheral CLOCKs, which are located in the adrenal glands and other components of the HPA axis and are regulated by the central CLOCK through neural and humoral pathways, also contribute to the rhythmic glucocorticoid secretion from these organs. Secreted glucocorticoids in turn reset and phase-delay circadian rhythm of the peripheral CLOCKs by stimulating the expression of several CLOCK-related genes. The peripheral CLOCKs also regulate glucocorticoid effect in local tissues through interaction between Clock/Bmal1 and GR, providing a local counter regulatory feedback loop to the effect of central CLOCK on the HPA axis.

ACTH: adrenocorticotropic hormone, AVP: arginine vasopressin, CRH: corticotropin-releasing hormone, PVN: paraventricular nucleus, SCN: Suprachiasmatic nucleus. Modified from (Nader et al., 2010).