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Stress and glucocorticoid receptor transcriptional programming in time and space: Implications for the brain–gut axis

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Abstract

Background—Chronic psychological stress is associated with enhanced abdominal pain and altered intestinal barrier function that may result from a perturbation in the hypothalamic–pituitary–adrenal (HPA) axis. The glucocorticoid receptor (GR) exploits diverse mechanisms to activate or suppress congeneric gene expression, with regulatory variation associated with stress-related disorders in psychiatry and gastroenterology.

Purpose—During acute and chronic stress, corticotropin-releasing hormone (CRH) drives secretion of adrenocorticotropic hormone (ACTH) from the pituitary, ultimately leading to the release of cortisol (human) and corticosterone (rodent) from the adrenal glands. Cortisol binds with the GR in the cytosol, translocates to the nucleus, and activates the *NR3C1* (nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)) gene. This review focuses on the rapidly developing observations that cortisol is responsible for driving circadian and ultradian bursts of transcriptional activity in the *CLOCK* (clock circadian regulator) and *PER* (period circadian clock 1) gene families, and this rhythm is disrupted in major depressive disorder, bipolar disorder, and stress-related gastrointestinal and immune disorders. GR regulates different sets of transcripts in a tissue-specific manner, through pulsatile waves of gene expression that includes occupancy of glucocorticoid response elements located within constitutively open spatial domains in chromatin. Emerging evidence supports a potentially pivotal role for epigenetic regulation of how GR interacts with other chromatin regulators to control the expression of its target genes. Dysregulation of the central and peripheral GR regulome has potentially significant consequences for stress-related disorders affecting the brain–gut axis.

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Conflicts of Interest

None

Keywords

chronic stress; CLOCK genes; epigenomics; intestinal barrier function; irritable bowel syndrome; mental health disorders

Background

The human glucocorticoid receptor α isoform, herein referred to as GR, is responsible for the maintenance of physiological homeostasis. GR has been implicated in the etiology of a wide range of stress-related disorders. Molecular mechanisms underlying GR gene expression are widespread and diverse in the human genome. They include opportunistic nucleosome occupancy, preprogramming of chromatin to permit indiscriminate binding of steroid receptors, and distal enhancer-promoter looping to transcriptionally poised genes (1, 2, 3, 4, 5, 6, 7, 8, 9). The GR controls target genes through long-distance looping to promoters within the three-dimensional milieu of euchromatin (10, 11, 12). A range of cooperative interactions with trophic factors, coactivators, corepressors, and other nuclear receptors, including brain-derived neurotrophic factor (BDNF), are important in GR regulation (13). Although greater than 1% of all transcripts in the human genome are regulated by the GR, circumscribed cell- and tissue-type expression is a prominent characteristic of glucocorticoid regulation.

Allele-specific bias is not uncommon among genes that are glucocorticoid responsive, including asymmetric expression of targets resulting from allele-specific methylation and parent-of-origin of monoallelic effects (14). In brain, studies in humans and in rodent models show that chronic stress and early life adversity disrupts normal ultradian GR pulsatile chromatin interactions during critical periods of development in an allele-specific manner. This stress-induced disruption has been associated with psychiatric disorders which persists and may be transmitted through maternal or paternal alleles across generations (15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26). Similarly, chronic stress and altered regulation of GR expression and function have been implicated in irritable bowel syndrome, intestinal barrier dysfunction, inflammatory bowel diseases, and alterations in the gut microbiome (27, 28, 29, 30).

Epigenetic modifications are known to play an important role in chronic stress–associated altered gene transcription (31). Epigenetics refers to external modifications to DNA and histones affecting gene transcription that are independent of DNA sequence, but are often driven by DNA sequence variation. One example of an epigenetic change is DNA methylation, which involves the addition of a methyl group to specific DNA sites, thereby preventing the expression of specific genes. Another example is histone modification. DNA wraps around histone proteins to form compact DNA–histone complexes. Modifications that relax the DNA–histone compaction state, such as acetylation at specific histone sites, allow accessibility to proteins that “read” genes and, thereby, promote gene transcription (32).

Insights from the central nervous system and psychiatry

Expression quantitative trait loci (eQTLs) are genomic loci that regulate gene expression. eQTLs may act in cis (locally) or trans (at a distance) to a gene. Cis-eQTLs, methylation-quantitative trait loci (meQTLs), and QTLs associated with the histone mark H3K27ac, which indicates enhancer and promoter sites of active gene regulation, are significantly enriched in domains containing the GR response element (GRE) (33). These domains are targeted by cis-regulatory, transcription factor–enriched sites within the human *NR3C1* gene that contain individual SNPs, and elsewhere, depending on cell type (3, 34). Chromatin remodeling, which can be observed near GR-regulated genes using live cell imaging immediately after treatment with the synthetic glucocorticoid dexamethasone, occurs with a well-defined ultradian rhythm (6, 9, 35). Numerous studies have shown that loss of data about the chromatin environment of the human *NR3C1* gene limits understanding of the clinical consequences of genetic variation (36, 37, 38). In the context of genetic association studies, one confounding problem has been the influence of population structure, as the allele frequency of a given variant, but also haplotype structure, varies widely depending on ethnicity (39).

Most studies show that abuse, neglect, and/or lack of parenting during early childhood are associated with blunted cortisol cycling in adolescence (21, 40, 41); although the directionality of effect is still controversial as a consequence of variability in study designs (42). Blunted cortisol and ACTH responses are associated with major depressive disorder, especially in females (43). In humans, chronic stress of the mother while the infant is *in utero*, and early life stress between birth and 5 years of age has been shown to affect DNA methylation of specific sites within the *NR3C1* gene (25, 26, 44). It has been proposed that there is a critical period during child development when abuse and trauma can exert an irreversible lifelong and transgenerational impact (24). Hyper-suppression of plasma cortisol is a feature of adult patients with major depressive disorder and/or posttraumatic stress disorder who have been abused as children. The flattening of the normal circadian cortisol curve in women who were abused as children suggests a mechanism that involves genomic variants in critical genes that govern *NR3C1* gene expression interacting with epigenetic modifications to disrupt regulated GR periodicity. For example, data suggest that early childhood trauma interacts with a risk allele in the *FKBP5* (FK506 binding protein 5) gene that encodes a molecular chaperone of the GR. Early childhood stress caused by sexual or physical abuse or neglect, especially between the ages of 0–5 years, results in demethylation of CpG sites within intron 7 of the *FKBP5* gene, leading to overexpression of *FKBP5*, GR resistance, and dysregulation of the central GR regulome (14). This may lead to flattening of the diurnal plasma cortisol curve in such individuals and put them at greater risk of developing major depressive disorder, posttraumatic stress, and functional bowel disorders later in life. Further research is needed to understand the molecular substrate of these phenomena.

Rhythmic patterning of gene expression

The GR is the major central nervous system determinant of metabolic rhythmicity in humans. Through the hypothalamic–pituitary–adrenal (HPA) axis, the GR regulates

peripheral glucocorticoids, such as cortisol (45, 46). Mouse and human studies show that about 10% of all genes exhibit diurnal rhythmicity. Neurons of the suprachiasmatic nucleus of the hypothalamus are the only cell type known to exhibit endogenous circadian rhythmicity in the absence of all inputs (47), although when these neurons are dissociated in mutant Cry1 −/− knockout mice, their endogenous rhythms become disrupted and asynchronous. In peripheral tissues, inputs such as light, temperature, and metabolic signaling reset various rhythms (48, 49, 50, 51, 52, 53). Peripheral tissue clocks regulate a variety of metabolic processes, including lipogenesis, insulin secretion, glucose clearance, lipid accumulation, and food absorption (49). Circadian clocks are based on feedback loops that involve a heterodimer of the transcription factors CLOCK (circadian locomotor output cycles kaput) and brain and muscle ARNTL (aryl hydrocarbon receptor nuclear translocator–like; formally known as brain and muscle ARNT-like 1 [BMAL1]), which program transcriptional dynamics of rhythmicity, including those of the period (*PER1*, *PER2*, and *PER3*) and cryptochrome (*CRY1* and *CRY2*) gene families. Maintenance of the circadian and ultradian rhythmicity of cortisol release appears to be regulated through acetylation and deacetylation of the GR by the *CLOCK* gene (46, 54, 55), and through *CRY1* and *CRY2* rhythmic repression of GR expression in tissues, such as the liver (56). Disruption of the periodicity of GR expression is associated with disease states characterized by glucocorticoid resistance or sensitivity (57, 58). The periodicity of GR function is important based on two observations: 1. During acute physiological states, such as the stress response, the need for rapid transactivation or transrepression of GR-regulated gene expression, mediated by the HPA axis, may explain the presence of preassembled, spatial chromatin domains where GR can bind in seconds (6, 9, 12, 33, 37, 38, 57, 59, 60, 61), and 2. Cortisol release exhibits both ultradian and circadian rhythmicity, and the temporal dynamics of GR binding to nuclear sites follows the circadian cycle as visualized using microscopy in living cells (6, 9, 61). GR chromatin immunoprecipitation sequencing (ChIP-Seq) studies confirm that the GR occupies existing DNase I hypersensitive sites, allowing for rapid binding to GREs and inverted repeat negative GREs (62). DNase I hypersensitivity indicates open chromatin, and is a primary indicator of sites of transcriptional regulation. Although the GR initiates the opening of closed chromatin, along with the recruitment of other factors that can assist in “tethered” binding, similar to the androgen receptor (63), it also interacts with specific transcripts that are transcriptionally poised, acting through long-distance “looping” (11, 12).

Recent studies show that chronic exposure to cortisol in human cell lines or corticosterone in mouse mammary epithelial cells unmasks GR-binding sites not evident after pulsatile steroid exposure (64). This may help explain why chronic stress in humans, which is accompanied by continuous cortisol secretion, acts to blunt healthy cortisol-mediated GR responses later in life, compared to pulsatile increases in hormone levels in blood that are a normal component of the acute stress response (65, 66, 67). Thus, in a murine cell model involving a 60-min exposure to corticosterone, ChIP-Seq demonstrated the presence of thousands of unique GR-binding sites, not found after 60-min pulses of hormone (64). Additionally, the Per1 transcription factor was more significantly associated with chronic exposure than Hes1 (Hes family bHLH transcription factor 1), which was associated with pulsatile exposure

(64). In humans, this may be related to the disruption of circadian, ultradian, and seasonal sleep habits associated with a variety of diseases.

The complexity of *NR3C1* gene transcriptional regulation

The *NR3C1* promoter lacks a consensus TATA box and CCAAT motif, although it contains binding sites for transcription factors, such as AP1 (activator protein 1), SP1 (specificity protein 1), nuclear factor-κ (NFKB1), CREB (cAMP response element–binding protein), EGR1/NGF1A (early growth response 1/nerve growth factor 1), and others. The *CLOCK* gene has inherent histone acetyltransferase activity, and acetylates lysine moieties located within the hinge region of the GR, a lysine cluster containing a KXKK motif, and represses *NR3C1* transcriptional activity with a diurnal rhythm (46, 54, 55). Neurotrophins, such as NGF (nerve growth factor) and BDNF (brain-derived neurotrophic factor), also regulate *NR3C1* transcriptional activity, at least in animal models. For example, rat cortical neurons treated with both BDNF and dexamethasone produced a unique set of GR-regulated genes associated with neuronal growth and differentiation (13). The BDNF receptor NTRK2 (neurotrophic tyrosine kinase, receptor, type 2) increased GR induction of these genes, whereas BDNF has been shown to phosphorylate serine within the N-terminus of the GR, increasing nucleosome occupancy and cofactor recruitment at glucocorticoid binding sites (13). Methylation of the exon 1F promoter of the *NR3C1* gene is variable, but hyper-methylation is associated with: 1. blunted cortisol response in adults who have experienced childhood loss (68), 2. increased salivary cortisol response in the children of mothers who were depressed during pregnancy and, 3. late gestational age if this methylation occurs in the fetus (69). Hyper-methylation of *NR3C1* has also been found in postmortem brain tissue of suicide victims with a history of child abuse (70, 71). Ultra-rapid negative feedback of *NR3C1* expression is mediated by FKBP5, which can lead to sustained repression of *NR3C1* expression through hyper-methylation of the 1F promoter, concomitant with abnormal disease states associated with glucocorticoid resistance (72, 73). A functional nGRE in exon 6 of the *NR3C1* gene and a long-range interaction occurring between this intragenic response element and the transcription start site are instrumental in this repression. This auto-regulatory mechanism of repression shows that the GR concentration can coordinate repression with excess ligand, regardless of the combinatorial associations of tissue-specific transcription factors. Consequently, the chronic nature of inflammatory conditions involving long-term glucocorticoid administration may lead to constitutive repression of *NR3C1* gene transcription, and thus to glucocorticoid resistance. This is one of several long-distance, epigenetic mechanisms used to stop expression of *NR3C1* and its target genes (10, 11, 12).

Chromatin dynamics

Upon entry into the nucleus, the GR diffuses throughout chromatin and opportunistically finds poised spatial domains of open chromatin, as defined by DNase I hypersensitivity and chromatin conformation capture (5, 8, 12). The GR also modifies chromatin in these domains, rapidly cycling in and out of GREs, and recruiting other cofactors (59, 60). Trans-repression can be exerted by three mechanisms: 1. through “negative” palindromic GREs characterized by inverted repeats (IR nGREs), which act by direct binding of glucocorticoid agonist–liganded GR, producing a silencing repressor complex through the association of

NCOR2 (nuclear receptor corepressor 2) complex corepressors and histone deacetylases (7), 2. the GR forms complexes with molecules, such as histone H3 lysine 9 methyltransferase G9a, which apparently can act as a molecular scaffold in modulating the periodicity of a subset of GR-regulated genes (74), and 3. indirect suppression using corepressors, such as GRIP1 (75). Euchromatin is characterized by DNase 1 hypersensitivity and specific combinations of histone marks that define active genomic regulatory elements, such as promoters H3K4me3 and H3K27ac, and enhancers H3K4me1 and H3K27ac. An enhancer can either increase or decrease transcription. Recent research demonstrates that, in brain, the DNA sequence CAC is a common site of methylation, in contrast to other tissues, where CpG is most often methylated (76). Following activation by endogenous cortisol in humans or by exogenous glucocorticoids, such as dexamethasone, GR protein is rapidly expressed and binds to molecular co-chaperones, such as heat shock protein (hsp90), p23 (prostaglandin E synthase 3-cytosolic), and the immunophilin FKBP5, which are important for its translocation to the nucleus. GR protein also participates in a process called assisted loading at some sites in chromatin (61), where it transiently increases accessibility to closed nucleosomes, enabling other nuclear receptors, such as the estrogen receptor, to bind at the same GRE, in what has been called a hit-and-run cycle (5, 9). Research has shown that GR binding to open chromatin, as defined by DNase I hypersensitivity, can be defined as: 1. opportunistic binding to spatial clusters of constitutively open chromatin that can also be used by other transcription factors, 2. GR binding to transcriptionally poised genes and transcripts located within already DNase I–hypersensitive sites, and 3. reprogramming of closed chromatin to open chromatin by activated GR bound to glucocorticoid, which probably occurs at the most conserved GRE motifs (62, 77). The transcription factor AP1 is present at all of these GRE-binding sites (77).

Long-range interactions that control GR-regulated genes by three-dimensional looping

The architecture of the three-dimensional genome provides the basis for the regulation of gene expression, bringing enhancers and promoters into close proximity, although they may be far apart in linear sequence (78). Thousands of significant long-range looping interactions have been found to link gene promoters and distal loci, reinforcing the notion that many, if not all, gene promoters engage with distal elements using a three-dimensional loop of chromatin that confers spatial proximity, as detected by chromatin conformation capture (10). A common class of long-range interactions mediated by the GR involves the looping of promoters to sites bound by the insulator protein CTCF, the CCCTC-binding factor (zinc finger protein) whose gene (*CTCF*) is not only highly regulated by glucocorticoids (11, 12) but exhibits significant allele-specific methylation in humans (79, 80, 81, 82, 83, 84, 85, 86). One example of glucocorticoid-mediated looping is trans-repression of the *NR3C1* gene through intragenic auto-regulation (4). In some examples of tissue-specific transcription induced by glucocorticoids, there is evidence that looping between GREs and their target genes takes place in a hormone-independent manner. For example, using associated chromatin trapping, lipocalin 2 (*LCN2*) was found to be looped to a GRE upstream of the Cip1-interacting zinc finger protein (*CIZ1*) gene prior to treatment with dexamethasone,

although both genes are regulated by the GR (12). The loop structure is absent in pituitary (AtT-20) cells, in which glucocorticoid induction of these genes does not occur (12).

Cell and tissue-specific regulation of target genes by the GR

There is no distinct set of GR-regulated genes in the human genome; instead, results from many studies show that these genes tend to be tissue-specific, at least in human and animal cell lines (87, 88). Although some studies have attempted to define all of the GR-regulated or glucocorticoid-sensitive genes or transcripts in the whole genome, detailed comparison of GR-regulated genes from different tissues, species, and cell types shows negligible overlap. However, there is considerable evidence from both rodent and human studies that *CLOCK*, *PER*, and *CRY* gene families are highly regulated by nuclear receptors and constitute part of a master transcriptional system of peripheral circadian rhythmicity in humans.

The GR regulates the expression of restricted sets of tissue-specific transcripts in mouse and rat hippocampus, dorsal root ganglion (DRG) neurons, human brain, human liver, and various cell lines, as well as differences following maternal care in the mouse Nr3c1 gene in different tissues and cell types, as determined by assessing DNA methylation by sequencing. For example, in liver, Grøntved *et al* (88) showed that for both mouse and human, C/EBP (CCAAT/enhancer–binding protein) maintains chromatin accessibility and enables GR recruitment to GREs and IR GREs in most GR-binding sites. C/EBP is highly regulated by glucocorticoids in liver but not brain (87, 88). The ubiquitous CTCF insulator, sometimes acting in concert with cohesion, broadly defines the boundaries of specific gene bodies, as well as topologically associated domains (TADs), which may be regulated by an interaction between the GR and transcriptionally poised genes, similar to enhancer-regulated genes (11). In addition, certain genes, such as MMTV (mouse mammary tumor virus) and FKBP5 are transcriptionally poised for specific GR control, fitting into a model of tissue-specific gene expression (11, 89, 90, 91, 92).

In the mammalian central nervous system, responsiveness to glucocorticoids appears to be mediated by both the mineralocorticoid receptor and the GR in some cells of the hippocampus (93). However, although the mineralocorticoid receptor is expressed in many tissues, such as the kidney, colon, heart, hippocampal formation, brown adipose tissue, and sweat glands, its expression is often protected from glucocorticoid activation through co-localization of HSD11K (11-beta-dehydrogenase isozyme 2), a GR-regulated gene, whose product inactivates cortisol to cortisone (94, 95, 96, 97, 98). In the context of inflammatory disease, GR acts in a reciprocal manner with NFKB1, a phenomenon that has been well-characterized; although a genome-wide coactivation analysis shows that GRE and NFKB1 bind only in a fraction of GR- and p65-(NF-κβ protein 65) binding sites (78). Studies of neuronal PC-12 cells suggest that greater than 40% of GR-binding sites are not GREs, as defined by the canonical motif, as well as related motifs, consisting of A/G-G—ACA---T/A-GT-CTC (87). Of the 1031 sites that bound the GR, only 25 were shared with 3,857 GR-binding sites in A549 cells derived from human lung epithelium, and 73 shared with the 8,265 GR-binding sites found in the mouse adipocyte 3T3-L1 cell line (8, 87).

Cooperativity, cross talk, pioneer molecules, and transcription factors

As described above, pleiotropy and promiscuity are prominent characteristics of GR interactions with chromatin, and the subsequent regulation of gene expression. Other steroid receptors, such as the estrogen receptor, benefit from GR reprogramming of chromatin in a cell type–specific manner. For example, in a mouse mammary tumor cell line designed to exclude progesterone receptor cross talk, ChiP-Seq analysis shows that priming with dexamethasone treatment significantly increases the binding of estrogen receptor to sites within DNase-I–accessible chromatin compared to treatment with estradiol alone, suggesting that glucocorticoids provide accessibility to estrogen receptor–binding sites that are only accessible after GR programming (38). Specific cross talk between NFKB1 and GR involves significant reprogramming of gene expression under conditions when both tumor necrosis factor (TNF) and glucocorticoids are present, lending support to the notion that GR and p65 are recruited by each other, and probably by other factors, such as AP1, to binding sites gained on coactivation, in a mutually dependent manner (78). At many genomic sites where GR binding causes increased chromatin accessibility, concurrent steady-state binding levels for another receptor are actually increased (61). Thus, although GR occupancy of a GRE may be short-lived, it acts cooperatively through assisted loading to enable GRE binding for the estrogen receptor at the same site (61). The mechanisms by which the GR enables other transcription factors to bind, resulting in either a positive or negative transcriptional outcome at composite response elements, has been extensively studied using the MMTV promoter as a model (99, 100, 101). In the central nervous system, CRH expressed by neurons of the paraventricular nucleus of the hypothalamus is critical for the release of ACTH from the anterior pituitary, and the subsequent release of cortisol from the adrenal glands. At the CRH promoter in these neurons, glucocorticoid-dependent negative feedback regulation, which is critical for limitation of the HPA-mediated stress response, is controlled by a composite element encompassing high-affinity binding sites for AP1 and GR at adjacent elements within the nGRE. CRH trans-repression by glucocorticoids involves formation of a repressor complex consisting of GR, MECP2 (methyl CpG binding protein 2), and HDAC1 (histone deacetylase 1), as well as recruitment of DNMT3b and associated changes in proximal promoter methylation (102). GR trans-repression is known to act through intermediary transcription factors that impact the nervous and immune systems, including NFKB1 (nuclear factor NF-kappa-B p105 subunit), AP1, CREB1, NFATC1 (nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1), STAT6 (signal transducer and activator of transcription 6, interleukin-4 induced), IRF3 (interferon regulatory factor 3), STAT3 (signal transducer and activator of transcription 3), GATA3 (GATA binding protein 3), and TBX21 (T-box 21) (103). Transcription factors are also involved in extensive remodeling of nucleosome structure by the GR.

GR rhythmicity, dysregulation, and the brain–gut axis

The *CLOCK* gene, whose translational product has a helix–loop–helix structure, has a primary self-oscillatory component that drives circadian rhythm by acting by acetylating the hinge region of the GR (54, 55). This mechanism normally represses GR-regulated transcriptional activity, acting in a 180-degree phase shift relative to the HPA-mediated oscillatory cycle (46). Changes in this inverse relationship between HPA-mediated and

CLOCK cycling have been implicated in shift work disorders (63, 104), cardiovascular diseases such as hypertension (104), obesity associated with sleep deprivation (105), and a range of inflammatory disorders (106), including irritable bowel syndrome, gastroesophageal reflux disease, and peptic ulcer disease (107).

Dysregulation of the HPA-mediated stress response is critical to the decoupling of the phasing of GR-regulated and CLOCK-regulated gene expression in many psychiatric disorders (108, 109, 110, 111, 112, 113, 114, 115), although the molecular mechanisms that synchronize the periodicity of this relationship are not completely understood. Studies of the regulation of *PER2*, a clock-controlled gene, show a unique overlapping of GRE and E-box motifs in its proximal promoter. *PER2* responds rapidly to glucocorticoid-induction through a complex including ARNTL (aryl hydrocarbon receptor nuclear translocator–like protein), also known as brain and muscle ARNTL (BMAL1), and CLOCK, and is involved in delaying circadian rhythmicity mediated by the GR (116). However, many GR-regulated genes are expressed in a variety of different phases relative to the circadian cycle in the mouse (117). The circadian clock is regulated at the cellular level by a transcriptional feedback loop. CLOCK:ARNTL heterodimers activate the expression of the *PER* and *CRY* genes, acting as transcription factors directed to the PER and CRY promoters via E-box elements. PER and CRY proteins form heterodimers and suppress the activity of CLOCK:ARNTL, completing the feedback loop. In addition, ARNTL has been shown to directly mediate circadian control of protein translation (118).

It is largely unknown how chronic stress directly or indirectly affects GR expression and function in the GI tract. Chronic, intermittent water-avoidance stress (WAS), 1 h per day for 10 days, significantly reduces the levels of the GR and epithelial tight junction protein levels in the colon but not the jejunum (119). These changes correlate with increased permeability to low molecular weight molecules, and a significant increase in visceral pain in response to colorectal distension. GR appears to play a pivotal role in these responses based on the observation that the GR antagonist RU-486 significantly reversed chronic stress–associated decrease in epithelial tight junction protein levels, increased paracellular permeability, and visceral hyperalgesia. It is probably relevant that the baseline level of GR expression in healthy rats is markedly higher in the mucosa of the colon compared to the jejunum (119).

Ontogenetic maturation of a circadian clock from the fetal stage until weaning has been demonstrated in the rat colon (120). This study suggests that the molecular mechanism for entraining the circadian clock in the colon appears to involve maternal breast-feeding, but subsequently, a suprachiasmatic nucleus–independent developmental signal switches the colonic clock from a maternal-dependent to maternal-independent stage. Consistent with reports that cortisol is responsible for driving circadian surges of transcriptional activity in the *CLOCK* and *PER* gene families in the central nervous system, expression of the epithelial tight junction proteins occludin and claudin-1 are under circadian control in the mouse large intestine (121), which supports an earlier report that the circadian clock regulated intestinal permeability (122). The expression of occludin and claudin-1 in the large intestine epithelium demonstrated daily oscillations, which require normal Per2 activity. In addition, the temporal changes of the expression levels were inversely associated with colonic permeability and differential susceptibility to dextran sodium sulfate (DSS)–induced

colitis between wild-type mice and mice with a mutation of the clock gene Period 2 (Per2; mPer2(m/m)). Occludin and claudin-1 mRNAs showed daily oscillations; Clock and Arntl (Bmal1) bonded directly to the E-box elements of occludin and claudin-1 promoters; and overexpression of Clock and Arntl activated occludin and claudin-1 promoters containing E-box elements. These observations suggest that occludin and claudin-1 are CGs with E-box elements in the promoter region directly regulated by Clock:Arntl heterodimers. The study did not exclude the possibility that posttranscriptional mechanisms may also underlie the circadian regulation of occludin and claudin-1 expression. Colonic permeability was also under circadian control, which was inversely associated with occludin and claudin-1 expression levels. Of interest, IL-1β and TNF-α mRNA levels did not show circadian oscillations in wild-type mice, and were comparable between wild-type and mPer2m/m mice. Therefore, Clock:Arntl heterodimers probably regulate colonic permeability in a circadian manner by timing the expression levels of claudin-1 and occludin, thereby affecting tight junction function. It remains to be determined whether the changes in occludin and claudin-1 expression are primarily responsible for daily changes in tight junction permeability and susceptibility to DSS-induced colitis or, alternatively, the reduced susceptibility to DSS-induced colitis in mPer2m/m mice may be due to some type of immune cell dysfunction. Nevertheless, the chimeric mouse experiments suggest that the *Per2* mutation in non-hematopoietic cells is responsible for the protection against DSS-induced colitis.

Role of the GR in chronic stress-induced visceral hyperalgesia

Chronic stress is associated with down-regulation of the anti-nociceptive endocannabinoid CB1 receptor (*CNR1* gene) and upregulation of the pro-nociceptive endovanilloid TRPV1 receptor in the subpopulation of nociceptive DRG neurons innervating the pelvic organs, including the colon. The effect of chronic stress on both CB1 and TRPV1 receptor expression and function are prevented by treatment with RU-486, supporting an important role for GR in this pathway (123, 124). Subsequent observations support a potentially pivotal role for epigenetic regulatory pathways in chronic stress–induced visceral hyperalgesia (125). Male rats were subjected to WAS or subcutaneous injections of corticosterone that reproduced chronic stress levels. Lumbar 4–5 and lumbar 6–sacral 2 DRGs were collected and compared between stressed and sham-stressed control rats. L4–L5 DRG neurons contribute to the somatosensory innervation of the lower extremities by way of the sciatic nerve, whereas L6–S2 DRG neurons innervate pelvic organs, including the colon. DNA methylation was measured at genes encoding NR3C1 and CNR1. Protein levels of CNR1 and DNA (cytosine-5-)-methyltransferase 1 (DNMT1) were knocked down in DRG neurons *in situ* with small interfering RNAs. Visceral pain was measured in response to colorectal distention. Chronic stress was associated with upregulation of DNMT1-mediated methylation at CpG sites involving both NR3C1 and CNR1 promoter regions, resulting in downregulation of NR3C1-induced expression of CNR1 in L6–S2, but not L4–L5 DRGs. Gene silencing of DNMT1 in L6–S2 DRG neurons of rats reduced DNA methylation and prevented chronic stress–induced increases in visceral pain. These studies indicate that the NR3C1 receptor acts as a positive transcription factor on *CNR1* expression. Chronic stress increases DNA methylation at upstream CpG promoter sites for *NR3C1*

expression, resulting in decreased levels of the anti-nociceptive CNR1 receptor, culminating in enhanced visceral pain.

Chronic, intermittent stress also alters the level of NR3C1 in regions of the central nervous system involved in visceral pain registration. In the WAS rodent model, exposing the central nucleus of the amygdala (CeA) to elevated glucocorticoid levels was associated with significant reduction in NR3C1 receptor expression but no changes in mineralocorticoid receptor expression (126), which is consistent with findings from experiments in the hippocampus and studies using cell cultures (127). Epigenetic mechanisms appear to play a role in chronic stress–induced changes in NR3C1 receptor levels in the amygdala (128). Trichostatin A, a potent histone deacetylase inhibitor, significantly attenuated chronic stress–associated visceral hyperalgesia. Methylation of *NR3C1* was increased following WAS, which was associated with decreased *NR3C1* expression. In contrast, methylation of the corticotropin-releasing factor (CRF) promoter was decreased after WAS with a concomitant increase in CRF expression – an anticipated result. In a subsequent study, the authors examined the hypothesis that histone deacetylation contributes to the maintenance of chronic anxiety and pain induced by prolonged exposure of the CeA to corticosterone (129). Bilateral infusions of a histone deacetylase inhibitor into the CeA attenuated anxiety-like behavior, as well as somatic and visceral hypersensitivity resulting from elevated corticosterone exposure. Deacetylation of histone 3 lysine 9 (H3K9), through the coordinated action of the NAD^+-dependent protein deacetylase sirtuin-6 (SIRT6) and NFKB1-sequestered *NR3C1* expression, leading to disinhibition of CRF. These observations suggest that epigenetic programming in the amygdala, involving both methylation and histone modification, are important in the maintenance of chronic anxiety and pain.

Limitations of animal models

Much of what we know about how the GR regulates gene expression and behavior has been obtained from studies in rodents, with extension to human mechanisms of disease. However, recent studies show that although the complexity of GR interactions with the three-dimensional structure of programmed and un-programmed chromatin exhibits some degree of similarity between species, population differences in allele-specific and tissue-specific expression appear to be limited to humans (82). Programmed chromatin has been prepared by factors such as AP1, ER-alpha, or remodeling factors for subsequent transient occupation by the GR. Unprogrammed chromatin has not undergone this preparation for GR occupancy (88). In addition, rodents provide poor models of the human inflammatory response (130), and do not adequately represent higher cognitive contributions to stress-related psychiatric disease and ameliorative drug therapy (131, 132, 133). Studies from human cell lines underscore the inadequacy of the rodent to mirror the long-distance, enhancer-based regulation of GR transcription that is a defining characteristic of glucocorticoid regulation of gene expression in humans (1, 134, 135, 136, 137, 138, 139).

Summary

Diurnal rhythms in the HPA axis and chronic intermittent stress have differential, region-specific, and cell-specific effects involving the brain–gut axis, including intestinal barrier

function, peripheral pain signaling mechanisms, and central nervous system modulation of pain perception. It appears that epigenetic regulation of *NR3C1* expression plays a potentially key role in these actions. Epidemiological studies suggest that chronic stress–associated functional gastrointestinal disorders, such as irritable bowel syndrome, are more prevalent in females and individuals who have experienced significant stress early in life (140). Future studies will need to address the mechanistic basis for the distinct effects of gender and early life stress as predisposing risk factors in functional bowel disorders, and the potential role of stress-induced alterations in intestinal barrier function on visceral hyperalgesia. It is probable that alterations in rhythmic gene transcription, epigenetic regulation of gene transcription, and allele-specific gene expression play a significant role in the pathophysiology of functional gastrointestinal disorders that are characteristically inducible and reversible. These concepts are summarized in Figure 1.

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Key Messages

- Chronic stress is associated with the exacerbation of common mental health and gastrointestinal disorders, including depression and irritable bowel syndrome.
- Emerging evidence indicates that chronic stress alters physiological cortisol-mediated pulsatile (circadian and ultradian) gene transcription in a tissue-, cell- and allele-specific manner.
- Epigenetic studies indicate that chronic stress selectively alters both the DNA methylome and chromatin compaction state affecting gene transcription in central and peripheral pathways involved in a variety of important functions including mood and pain perception.
- Chronic and early life stress–associated disruption in glucocorticoid receptor–regulated gene transcription has a potentially significant impact on the brain–gut axis throughout the lifespan and across generations.

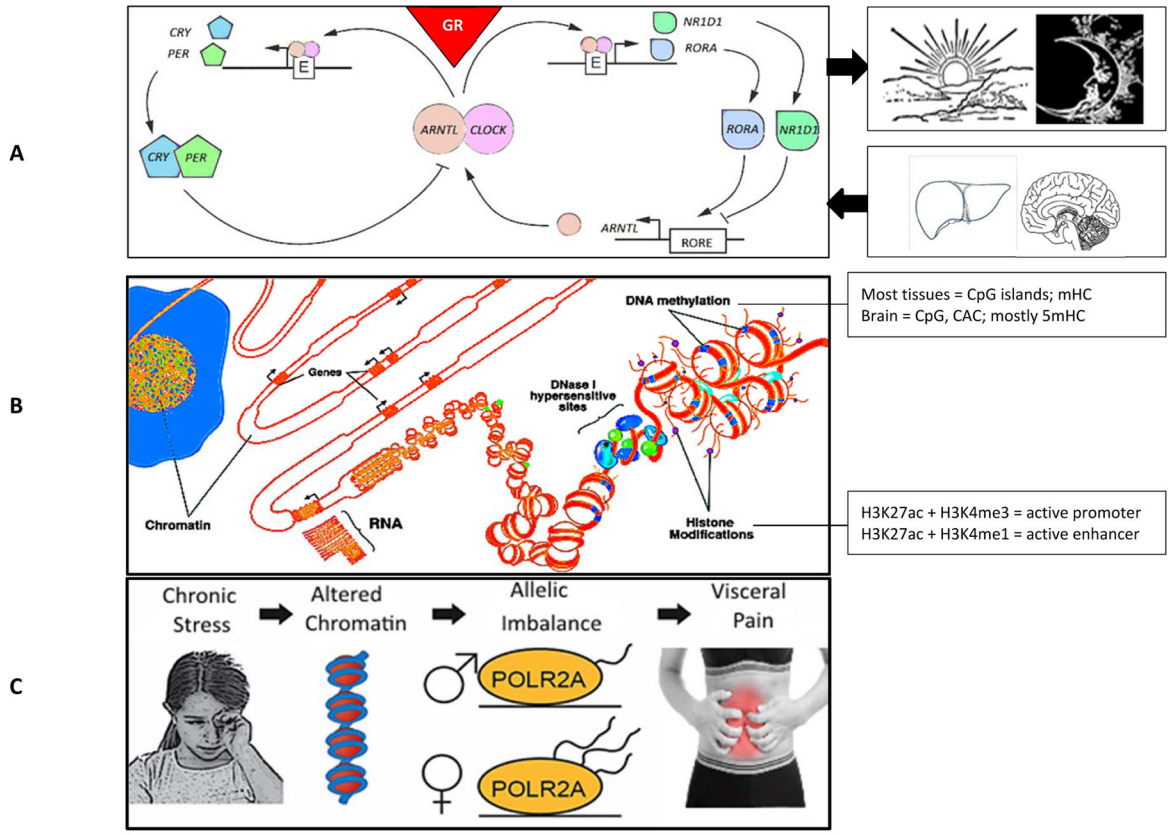

Figure 1.

The glucocorticoid receptor, regulation of gene expression, and its clinical consequences. (A) The glucocorticoid receptor (GR) and regulation of the *CLOCK* gene family. This pathway contributes to regulation of circadian rhythmicity, and is driven by the hypothalamic-pituitary-adrenal axis and metabolic inputs from liver. Of all the thousands of genes whose expression is regulated by the GR, the most highly regulated include members of the *CLOCK* gene family such as *PER1* (33). Products of *ARNTL* and *CLOCK* form a heterodimer that acts a transcription factor and binds to the E-box (E) in the promoter of other GR-regulated *CLOCK* and related genes, including *CRY*, *PER*, *NR1D1*, and *RORA*. In general, both *CRY* and *PER* genes are expressed in a circadian manner, acting either by repressing the CLOCK:ARNTL heterodimer, or blocking GR from binding to the GR response element (GRE) sequence. This latter mechanism is described in the section: GR rhythmicity, dysregulation, and the brain–gut axis. NR1D1 and RORA form a heterodimer that acts as a transcription factor that binds to the RORE sequence that is located in genes such as *ARNTL*. The ARNTL protein also acts independently of its role as a transcription factor to mediate translational control of specific proteins in a circadian manner (118). (B) Overview of results from the NIH Roadmap Epigenomics Mapping Consortium. Chromosomes are located in chromatin-bound territories in the nucleus. Euchromatin is characterized by DNase 1 hypersensitivity and specific combinations of histone marks that define active genomic regulatory elements, such as promoters H3K4me3 and H3K27ac, and enhancers H3K4me1 and H3K27ac. An enhancer can either increase or decrease transcription. Recent research demonstrates that, in brain, the DNA sequence CAC is a

common site of methylation, in contrast to other tissues where CpG is most often methylated. Also, in brain, 5-hydroxymethylcytosine (5hmC), a reactive species, is common. In contrast, in the periphery, methylcytosine (hmC) is common (76). (C) Chronic and/or early life stress appear to lead to alteration of chromatin and allele-biased gene expression, concomitant with psychiatric disorders and comorbid conditions including irritable bowel syndrome. In such cases, allele skewing of transcription (21, 22) is most likely a consequence of epigenetic alterations arising from environmental adversity that contributes to a variety of stress-related disorders distinct from parental imprinting (29, 30). POLR2A polymerase (RNA) II (DNA directed) polypeptide A, 220kDa is a highly conserved, ubiquitously expressed gene that encodes the largest subunit of RNA polymerase II, the polymerase responsible for synthesizing messenger RNA in eukaryotes and, therefore, plays an important role in gene transcription. Allelic imbalance refers to differential expression of the maternal vs paternal allele. POLR2A, DNA-dependent RNA polymerase II; H3K4me3, histone H3 trimethyl Lys4; H3K27ac, histone H3 Lys27 acetylation; H3Kme1, histone H3 monomethyl Lys4. of methylation, in contrast to other tissues where CpG is most often methylated. Also, in brain, 5-hydroxymethylcytosine (5hmC), a reactive species, is common. In contrast, in the periphery, methylcytosine (hmC) is common (76). (C) Chronic and/or early life stress appear to lead to alteration of chromatin and allele-biased gene expression, concomitant with psychiatric disorders and comorbid conditions including irritable bowel syndrome. In such cases, allele skewing of transcription (21, 22) is most likely a consequence of epigenetic alterations arising from environmental adversity that contributes to a variety of stress-related disorders distinct from parental imprinting (29, 30). POLR2A polymerase (RNA) II (DNA directed) polypeptide A, 220kDa is a highly conserved, ubiquitously expressed gene that encodes the largest subunit of RNA polymerase II, the polymerase responsible for synthesizing messenger RNA in eukaryotes and, therefore, plays an important role in gene transcription. Allelic imbalance refers to differential expression of the maternal vs paternal allele. POLR2A, DNA-dependent RNA polymerase II; H3K4me3, histone H3 trimethyl Lys4; H3K27ac, histone H3 Lys27 acetylation; H3Kme1, histone H3 monomethyl Lys4.