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MOLECULAR MECHANISMS OF GLUCOCORTICOID RECEPTOR SIGNALING

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Abstract This review highlights the most recent findings on the molecular mechanisms of the glucocorticoid receptor (GR). Most effects of glucocorticoids are mediated by the intracellular GR which is present in almost every tissue and controls transcriptional activation via direct and indirect mechanisms. Nevertheless the glucocorticoid responses are tissue –and gene– specific. GR associates selectively with corticosteroid ligands produced in the adrenal gland in response to changes of humoral homeostasis. Ligand interaction with GR promotes either GR binding to genomic glucocorticoid response elements, in turn modulating gene transcription, or interaction of GR monomers with other transcription factors activated by other signalling pathways leading to transrepression. The GR regulates a broad spectrum of physiological functions, including cell differentiation, metabolism and inflammatory responses. Thus, disruption or dysregulation of GR function will result in severe impairments in the maintenance of homeostasis and the control of adaptation to stress.

Key words: glucocorticoid, glucocorticoid receptor, stress hormones

Resumen ***Mecanismos moleculares de señalización del receptor de glucocorticoides***. Esta revisión destaca los más recientes hallazgos sobre los mecanismos moleculares del receptor de glucocorticoides (GR). La mayoría de los efectos de los glucocorticoides son mediados por los GR intracelulares presentes en casi todos los tejidos y controlan la activación transcripcional por mecanismos directos e indirectos. Las respuestas a los glucocorticoides son específicas para cada gen y tejido. Los GR se asocian en forma selectiva con ligandos producidos en la glándula adrenal, corticosteroides, en respuesta a cambios neuroendocrinos. La interacción del ligando con el GR promueve: a) la unión del GR a elementos genómicos de respuesta a glucocorticoides, modulando la transcripción; b) la interacción de monómeros del GR con otros factores de transcripción activados por otras vías, llevando a la transrepresión. El GR regula un amplio espectro de funciones fisiológicas, incluyendo la diferenciación celular y las respuestas metabólicas e inflamatorias. Así, la desregulación de la función del GR resulta en graves defectos en el mantenimiento de la homeostasis y el control de la adaptación al estrés.

Palabras clave: glucocorticoides, receptor de glucocorticoides, hormonas del estrés

In higher organisms, glucocorticoids synthesized by the adrenal cortex, play an important role in the adaptation to stressors and consecutive maintenance of internal homeostasis. The activation of the hypothalamic pituitary adrenal (HPA) axis by stressful situations –from emotional stress to infection–, leads to increase in plasma cortisol (in primates)/corticosterone (in rodents) concentrations, which in turn affect almost all physiological systems in the organism[1]. Besides the coordinated regulation of immune and neuronal responses, glucocorticoids regulate the endocrine system, in particular the HPA-axis, inhibiting corticotrophin releasing hormone (CRH), corticotrophin (ACTH) and consecutively their own synthesis that restores homeostasis. Glucocorticoids not only exert immunosuppressive and antiinflamatory actions, they also regulate cell-growth, bone-density, cardio-vascular function, metabolism, development and reproduction. Glucocorticoids have also an important impact in the brain on cognition, behaviour, mood and sleep[2-4].

At the molecular level, the action of adrenal steroids is mediated by the glucocorticoid receptor (GR), a nuclear hormone receptor belonging to the superfamily of ligand-activated transcription factors. Three different 3'-splice variant of the GR have been shown: GR-α, the active form, GR-β, an inhibitor of GR-α function and GR-P, thought to be an activator of GR-α[5]. GR is predominantly localized within the cytoplasm as part of a complex with heat shock proteins and immunophilins. However, a continuous shuttling of the GR between the two cellular compartments takes place. Upon ligand binding, GR dissociates from the complex, where heat shock protein 90 (HSP90) plays a central role, and undergoes a conformational change[6]. Consecutively GR translocates to the nucleus. Activated GR induces or represses gene transcription either as homodimers or in a monomeric form. Glucocorticoids

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can also bind to mineralocorticoid receptors even with a higher affinity than the binding to GR. Moreover, when both receptors are coexpressed, they can form heterodimers that can bind to DNA with high affinity exerting singular properties[7, 8].

Disregulation of the HPA axis that leads to excessive glucocorticoid secretion may have profound pathological effects in the organism. HPA dysfunction plays a critical role in the pathophysiology of mood disorders such as anxiety, depression and cognition impairment[2].

GR gene variants have been associated to pathological conditions. Several polymorphisms have been described for GR. Among others, the N363S and BcII polymorphisms are associated with hypersensitivity to glucocorticoids and with increased abdominal fat mass, and major depression. In contrast the ER22/23EK polymorphism is related to glucocorticoid resistance and recent studies associate it with an increased risk of major depression[9, 10]. FKBP5, a co-chaperone of HSP90, regulates GR sensitivity. GR has less affitiny for glucocorticoids when FKBP5 is bound to the receptor complex. Polymorphisms in this gene are associated with increased expression of FKBP5 following GR activation, leading to a decreased negative feedback of glucocorticoids. This prolonged elevation of cortisol might be a risk factor for stress-related psychiatric disorders[11]. On the other hand certain polymorphisms in the FKBP5 gene may hasten the onset of antidepressant treatment response[12]. Moreover, hypersecretion of corticosteroids due to excessive adrenocorticotropic hormone (ACTH) secretion from a pituitary adenoma causes Cushing's disease, a severe clinical condition characterized by abnormal fat deposition around the neck, thinning of the skin, osteoporosis, insulin resistance, dyslipidemia, myopathy, amenorrhea, hypertension, anxiety and depression[13, 14]. These complex disorders still represents a major challenge for the physician in terms of efficient treatment. Understanding the molecular mechanisms of GR function may help in the development of new drugs for the treatment of Cushing's disease, stress-related disorders and other pathologies where excessive levels of corticosteroids play a causal role[15].

In this review we will summarize recent findings related to the GR mediated molecular mechanisms of action (Fig. 1).

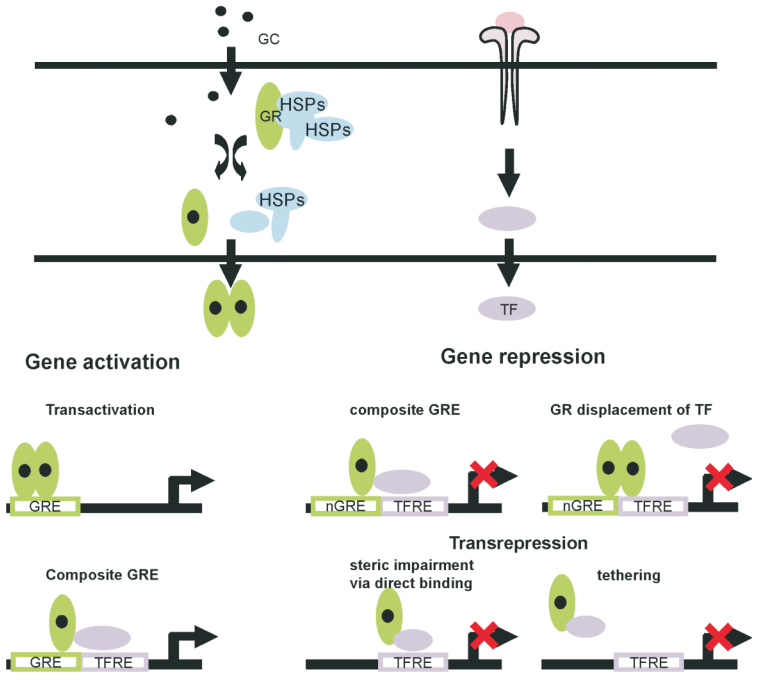

Fig. 1.– Transcriptional activities of GRs. Glucocorticoids can both activate and inhibit gene expression. Gene activation: GR homodimers bind to specific GRE in target genes and activate transcription. GR also contribute to gene activation by acting as coactivator for other TFs in composite GREs. Gene repression: several mechanisms of transcriptional repression have been documented for GR. By nGRE, GR displaces TFs or associates with them inhibiting gene transcription. By transrepression, GR inhibits the activity of TFs without DNA binding by GR (steric impairment or tethering). GC: glucocorticoids, GR: glucocorticoid receptor, GRE: glucocorticoid response element, nGRE: negative glucocorticoid response element, TF: transcription factor, TFRE: transcription factor response element, HSP: heat shock proteins. (The figure is presented in color in the web version of this article).

GR mediated transcriptional activation

Release of HSP90 allows GR translocation to the nucleus, homodimerization and DNA binding at glucocorticoid response elements (GREs) activating gene transcription (transactivation) in a cell and gene-specific manner[16]. GREs are palindromic sequences of two six base pair separated by a three base pairs spacer, present in the promoter of glucocorticoid-responsive genes. Deviation from this consensus sequence has been reported. Nevertheless GREs maintain important contacts with GR through specific functional groups on critical nucleotides within each palindromic sequence. Most of the genes mainly controlled by GR transactivation are involved in metabolic regulation; like increasing blood glucose levels, gluconeogenesis and mobilization of amino and fatty acids. When bound to DNA the glucocorticoid receptor interacts with transcriptional coactivator molecules, that stimulates transcription through direct interactions with the basal transcription machinery or by inducing local chromating remodelling, including histone acetylation or methylation, pointing to the roles of stress elicited glucocorticoids in mediating epigenetic modifications[17-19]. Some coactivators, like cyclic AMP response element binding protein (CBP) and p300 have histone acetyltransferase (HAT) activity and cause acetylation of core histones which facilitates the recruitment of RNA polymerase II and general transcription factors such as TATA box-binding protein (TBP)[20], resulting in gene activation. Other coactivators recruit chromatin modifying enzymes to allow promoter activation, such as the members of the p160 family of coactivators, including steroid receptor coactivator (SRC)-1 (NcoA1), SRC-2 (TIF-2, GRIP1) and SRC-3 (p/CIP, RAC3, ACTR or AIB1). These cofactors act as adaptor proteins linking the GR with other cofactors, like p300 and CBP, or with histone methyl transferases, like coactivator-associated arginine methyltransferase 1 and protein arginine methyltransferase 1[21].

Another common type of regulatory mechanism exerted by GR is their binding on composite GR-responsive regions, where additional transcription factors bind and efficiently induce glucocorticoid-mediated gene expression. Alternatively, the ligand-activated GR can modulate gene expression by interacting also with transcription factors, independently of binding to GREs. Transcription factors downstream to several signal transduction cascades, including cAMP response element-binding protein (CREB)[22], activating protein 1 (AP-1)[23, 24] and signal transducer and activator of transcription (STAT)[25, 26] are described to interact with activated GR and promote transcription. Although crosstalk between NF-κB and GR leads to a mutual inhibition (see below), in some cases, activation of both transcription factors results in a cooperative environment[27].

GR also recruits chromatin remodelling engines such as the mating-type switching/sucrose non-fermenting (SWI/SNF) complex. This complex is an ATP-dependent chromatin-remodeling factor with a multi-subunit structure[28]. It contains either BRG1 or hBrm as the central catalytic ATPase, as well as 10-12 BRG1-associated factors (BAFs). BRG1 alone can stimulate nucleosome remodelling, meanwhile addition of the core BAF subunits reconstitute chromatin remodelling to optimal levels[29]. SWI/SNF subunits can associate histone-modifying enzymes, transcription cofactors, tumor suppressor complexes and DNA replication factors. This ATP-dependent chromatin remodelling complex uses the energy derived from ATP hydrolysis to disrupt histone-DNA interactions in the context of GR activated transcription, thereby changing nucleosomal architecture leading to transcriptional activation. Although mainly related to transcriptional activation and considering that GRs can mediate either transcriptional activation or repression, it should be underlined that these enzymatic complexes have also been associated with transcriptional repression as demonstrated by activated corepressors[30].

GR mediated gene repression

As mentioned, GR can suppress gene transcription by different mechanisms. Transrepression[31, 32] accounts for many of the inhibitory effects glucocorticoids exert on the immune function and inflammatory processes[33-35]. In this mode of action, target genes are negatively regulated by GR via protein-protein interaction between GR and target transcription factors. This interaction may take place on promoters that do not contain GREs, with or without binding of the transcription factor to the DNA (steric impairment or tethering mechanism). Transrepression may take place on composite promoters as well, where GRE and transcription factors' responsive element may overlap. Thus, GR can interact with transcription factors like AP-1[36], NF-κB[37] and STAT and inhibits their activity without involving direct GR binding to the DNA. Interaction between GR and AP-1 or NF-κB at the promoters of transcriptionally activated proinflammatory genes, accounts for many of the anti-inflammatory actions of glucocorticoids. Interestingly, the interaction between GR and STAT family members results also in regulation of the immune system, leading to a synergistic enhancement or inhibition of the transcriptional activity depending on the cellular context[25]. Recently, it has been found that GR interacts with the Th1-specific transcription factor T-Bet, thereby inhibiting T-Bet activity implicated in Th1 cell differentiation and inflammation[38].

Corepressors, such as nuclear receptor corepressor (NcoR) and silencing mediator of retinoic acid and thyroid hormone receptor (SMRT), mediate gene repression in the

absence of ligand or presence of antagonist by interacting with histone deacetylases (HDACs) and thereby compacting chromatin. Activated GR binds to coactivators to inhibit HAT activity directly and recruiting HDAC2, which reverses histone acetylation leading to suppression of activated inflammatory genes. Moreover, recently it was described that HDACs also deacetylate nonhistone proteins and HATs acetylates them. As other nuclear receptors, GR is acetylated upon binding of corticosteroid to GR. Consequently acetylated GRs translocate to the nucleus, bind GRE and activate genes. HDAC2 deacetylate the acetylated GR, which, for instance, enables GR to repress NF-kB activation of regulated inflammatory genes[39].

Cofactors may change their function from being a coactivator to a corepressor. GRIP1 acts as a corepressor in GR-dependent regulation of AP-1 driven gene expression[40]. In the same direction, HDAC1 acts as a coactivator for the GR on the MMTV promoter[41]. Moreover, cofactor competition between GR and proinflammatory transcription factors have been described[42].

Thus, depending on the gene, the transcription factor composition or cellular context, the gene transcription outcome may be of an inhibitory or stimulatory nature.

Alternatively GR can interact with negative GREs[43] preventing transcription factors to bind to their binding site. Negative GREs are by far less frequent than positive GREs. At the hypothalamic and pituitary levels, corticotrophin-releasing hormone (CRH) and ACTH gene transcription are downregulated by negative GREs present in their promoters[44, 45]. However the molecular mechanism of GR mediated repression on these two gene targets seems to be different. Studies in GR mutant mouse models carrying a point mutation in the GR at the dimerization interface[33], suggest that only the repression of pro-opiomelanocortin (POMC, gene coding for ACTH) in the anterior pituitary is dependent of GR dimerization. Monomeric GR still repress CRH gene expression, probably through protein-protein interaction with transcription factors present in that promoter, like AP-1, CREB and Nurr77. Thus, a monomeric form rather than GR homodimers would block the activity of DNA-bound transcription factors without contacting the DNA itself [46]. Thus, even in the presence of negative GREs, GR can operate via different mechanisms, depending on the gene or tissue.

Inhibition of a target gene by GR may also alternatively take place at other regulatory points of signaling cascade. For instance, kinase-activating cascade, including MAPKs (p38, ERK, JNK) phosphorylates and activates inflammatory signals, like AP-1 or NF-κB target genes. However, all MAPKs have been identified as potential targets for activated GR through blockade of their activating phosphorylations. A novel aspect of GC transrepression has been shown by Beck et al [47]. GR blocks NF-κB transcriptional activity, by upregulating IκBα, the cytoplasmatic NF-κB inhibitor[48,49]. In addition to the well described interaction-based mutual repression mechanism between the GR and NF-κB, additional mechanisms were recently discovered. It was shown the GR recruits the nuclear NF-κB kinase, mitogen- and stress-activated protein kinase-1 (MSK1) to the cytoplasm, thus modulating the chromatin environment and function of the inflammatory enhanceosoma. Loss of MSK1 at inflammatory gene promoter, causes inhibition of NF-kB transactivation[47].

Modulation of GR by RNAs

More recently expanding numbers of noncoding RNAs (ncRNAs) with regulatory functions have been reported[50]. For example, growth arrest-specific 5 (Gas5), a single-strand ncRNA, was shown to interact with activated GR and suppressed GR-induced transcriptional activity of glucocorticoid-responsive genes by inhibiting binding of GR to target genes' GREs[51]. On the other hand, SRA (steroid receptor RNA activator) was reported to co-activate steroid receptors as an RNA transcript. It was shown that, even in the presence of cycloheximide, a protein synthesis inhibitor, transfected SRA enhanced endogenous GR activity in HeLa cells indicating that SRA acts as an RNA molecule rather than a protein[52]. These results have introduced a novel concept of regulation in nuclear receptor-mediated transcription and serve as another example of RNA transacted genomic information. Future studies will define more precisely the functional and physiological significance of RNA transcripts as components of protein complexes regulating transcription.

We conclude that the impact of glucocorticoids depends not only on the interplay among GR and DNA-regulatory elements, transcription factors, cofactors and chromatin remodelling complexes but also on the target gene and tissue. Moreover, signalling components of different signal transduction pathways originated from different stimuli, crosstalk, thereby increasing the level of complexity of gene regulation. Understanding the molecular mechanisms underlying glucocorticoids actions in the context of an integrated view of gene regulatory processes is necessary in order to design effective therapeutic strategies.

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Writing papers or books was also agonizing. The light style of some of my writings is misleading. George Wald hit it right when he once said: "This paper of yours is so lightly written that you must have sweated terribly." It was not always so. When I was younger I wrote more easily. Now it is hard work and I cannot decide whether my mind got weaker or my self-criticism stronger, or both. Now I have to rewrite anything I write five times or more. I am not the only one who must do so. The researcher who wrote the clearest papers was 0. Warburg. I asked him for his secret. "I rewrite sixteen times," he said. ["] *When I write first, I write up everything that comes to my mind. Then I put the paper away and rewrite a month later without looking at my first text. If the second text is different from the first, then I rewrite again. So I may rewrite sixteen times, till the text does not change any more*["].

Escribir artículos o libros fue también una agonía. El estilo ágil de algunos de mis escritos engaña. George Wald dio en el clavo cuando una vez me dijo: "Este artículo suyo está escrito tan ágilmente que usted debió sudar bastante". No siempre fue así. Cuando yo era más joven escribía más fácilmente. Ahora es un duro trabajo y no puedo decidir si mi mente se debilitó o si mi autocrítica se fortaleció. Ahora tengo que rescribir todo lo que escribo cinco veces o más. No soy el único que debe hacerlo. El investigador que escribió los artículos más claros fue O. Warburg. Le pregunté su secreto. "Yo rescribo dieciséis veces", dijo. ["] La primera vez, escribo todo lo que me viene a la mente. Luego dejo el escrito y lo escribo después de un mes sin haber visto el primer texto. Si el segundo texto es diferente al primero, lo escribo de nuevo. Así puedo rescribirlo dieciséis veces, hasta que el texto no cambia más["].

Albert Szent-Györgyi (1893-1986)

Looking Back. Perspectives in Biology and Medicine 1971; 15: 2.