Rev Endocr Metab Disord (2007) 8:321–330
DOI 10.1007/s11154-007-9059-8

Glucocorticoid receptor physiology

Marjet D. Heitzer · Irene M. Wolf · Edwin R. Sanchez · Selma F. Witchel · Donald B. DeFranco

Published online: 30 November 2007

Abstract Glucocorticoid action in cells is mediated by a specific receptor protein, the glucocorticoid receptor (GR). GR is a member of a superfamily of ligand-inducible transcription factors that control a variety of physiological functions; such as, metabolism, development, and reproduction. Unliganded GR is predominantly localized within the cytoplasm but rapidly and efficiently translocates to the nucleus following hormone binding. This review will focus on the intracellular signaling pathway utilized by the GR including the mechanisms that control its intracellular trafficking, hormone binding and transcriptional regulation. Many receptor-interacting proteins are involved in distinct steps in GR signal transduction, each with a unique mechanism to regulate receptor action and providing potential drug targets for the manipulation of cellular responses to glucocorticoids.

M. D. Heitzer · I. M. Wolf · D. B. DeFranco (✉)
Department of Pharmacology,
University of Pittsburgh School of Medicine,
Pittsburgh, PA 15260, USA
e-mail: dod1@pitt.edu

S. F. Witchel
Division of Endocrinology, Department of Pediatrics,
Children's Hospital of Pittsburgh,
Pittsburgh, PA 15260, USA

I. M. Wolf · E. R. Sanchez
Department of Physiology and Pharmacology,
University of Toledo College of Medicine,
Toledo, OH 43614, USA

1 Introduction

In a clinical setting, glucocorticoids are widely used as anti-inflammatory agents to control both acute and chronic inflammation. Research on steroid hormones began in the late 1800s and flourished during the twentieth century. The prevailing dogma of the time insisted these hormones were involved in enzymatic processes of metabolism and rebuked the idea of hormones working through receptors. In the early 1960s, utilizing a tritiated form of estrogen, Elwood Jensen and colleagues demonstrated that a hormone can be taken up and retained by specific tissues, thus leading to the identification of the estrogen receptor [1].

In 1950, Edward Kendall, Tadeus Reichstein, and Philip Hench were awarded the Nobel Prize in Physiology or Medicine for their studies on the structure and physiological effects of glucocorticoids. Working independently, Kendall and Reichstein isolated and determined the chemical structure of cortisol. Dr. Hench administered cortisol to patients suffering from rheumatoid arthritis; thereupon, exposing glucocorticoids as effective therapeutic agents [2]. In 1966, Allan Munck first identified the receptor for glucocorticoids from his experiments on rat thymic lymphocyte cytosol [3]. Subsequently, Ron Evans and his colleagues cloned the receptor in 1985, igniting an explosion of molecular studies on the glucocorticoids receptor (GR) and its related family members, steroid receptors [4].

2 Steroid hormone receptors

Steroid receptors are a superfamily of ligand-inducible transcription factors that control a variety of physiological functions; such as, metabolism, development, and repro-

duction via their control of specific target gene transcription. Members of this superfamily include steroid activated GR, mineralocorticoid receptor (MR), progesterone receptor (PR), estrogen receptor (ER), and androgen receptor (AR). These receptors share a common structural organization consisting of several modulatory domains with a highly conserved, centrally localized zinc-finger DNA binding domain (DBD), a less-well conserved carboxyl-terminal ligand binding domain (LBD), and a divergent amino terminal domain (Fig. 1). The amino terminal domain (NTD) is variable among receptors with the exception of a region rich in negatively charged acidic amino acids. This region is known as either AF-1 (activation function-1) or tau-1, and its function in transcriptional regulation can be ligand independent. The AF-1 region has been shown to interact directly with basal transcriptional machinery and with many other cofactors that participate in transcriptional regulation; moreover, disruption of AF-1 decreases reporter gene expression [5, 6].

Close to the AF-1 region is the DBD characterized by eight cysteine residues tetrahedrally organized about two zinc atoms. These zinc fingers create a three dimensional configuration that allows the binding of the receptor to DNA. More specifically, the amino terminal zinc finger discriminates DNA response elements and the carboxyl terminal zinc finger and is necessary for receptor dimerization [7]. Adjacent to the DBD is a variable hinge region that often contains a constitutive nuclear location sequence (NL1). This hinge enables the receptor to bend or change conformation [8].

Finally, the highly conserved ligand-binding domain (LBD) is located at the carboxyl-terminal. Not only is this region essential for hormone binding, it performs other functions. The LBD aids in receptor dimerization and contains sequences for protein–protein interaction with heat shock protein 90 (HSP90). The GR-HSP90 interaction facilitates proper folding of the receptor and prevents the receptor from binding to DNA in the absence of hormone. HSP90 binding may mask a second nuclear localization sequence (NL2). With the addition of hormone, HSP90 is released allowing NL2 to assist in receptor translocation through the nuclear pore [9]. A second activation function (AF-2) domain is positioned at the LBD. AF-2 is hormone dependent and undergoes a conformational change that allows for the interaction of accessory factors that participate in transcriptional activation (i.e. co-activators) or repression (i.e. co-repressors) [10]. Furthermore, AF-2 can act synergistically with AF-1 to mediate transcriptional activity [11].

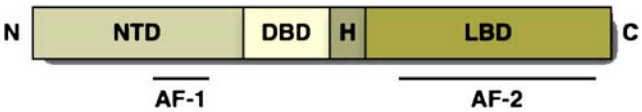

Fig. 1 General schematic of steroid receptor functional domains

3 Overview of GR signaling

Ligand binding initiates a process culminating in translocation of the ligand–receptor complex to the nucleus via the microtubule network (Fig. 2) [12]. Once in the nucleus, the activated GR associates with unique DNA target sties that are linked to hormone-regulated genes. GR can be recruited to target genes either through direct DNA binding (i.e. at elements termed glucocorticoid response elements or GREs) or through its interaction with other DNA bound transcription factors. The DNA sequences that GR recognizes at genes whose transcription will be activated by ligand-bound GR (i.e. receptor-dependent "transactivation") are related to a consensus palindromic sequence of two six base pair "half—sites" separated by a three base pair spacer. Natural gene sequences recognized by GR at such positive GREs can deviate from this consensus sequence but maintain important contacts with the receptor through specific functional groups on critical nucleotides within each half site. Once bound to such positive GREs as a homodimer, GR serves as a scaffold for the assembly of distinct macromolecular complexes that include coactivator proteins, chromatin remodeling factors and other factors that directly or indirectly engage the transcriptional machinery [13]. The genes mainly controlled by GR transactivation are involved in metabolic regulation; for example, increasing blood glucose levels, gluconeogenesis, and mobilization of amino and fatty acids [14]. Tyrosine aminotransferase (TAT) and phosphoenolpyruvate carboxykinase (PEPCK) are two key gluconeogenic enzymes that are typically used during *in vitro* and *in vivo* studies to measure GR transactivation efficiency [15].

The reduction of transcription (i.e. "transrepression") by GR occurs by different mechanisms. One mechanism resembles transactivation, but the receptors bind to DNA sequences distinct from positive GREs (i.e. negative GRE sites or nGREs) [16]. One aspect of negative feedback regulation of the HPA axis via repression of corticotropin releasing hormone (CRH) and adrenocorticotropic hormone (ACTH) involves GR repression of the proopiomelanocortin gene transcription through its interaction with nGREs linked to its promoter [17]. GR also triggers transcriptional repression through a mechanism that does not involve its direct DNA binding but rather a tethering to other DNA-bound transcription factors such as AP-1 and NF-κB [18, 19]. The anti-inflammatory actions attributed to glucocorticoids are mainly brought about through its interaction with AP-1 and NF-κB at the promoters of transcriptionally activated proinflammatory genes. Finally, while many

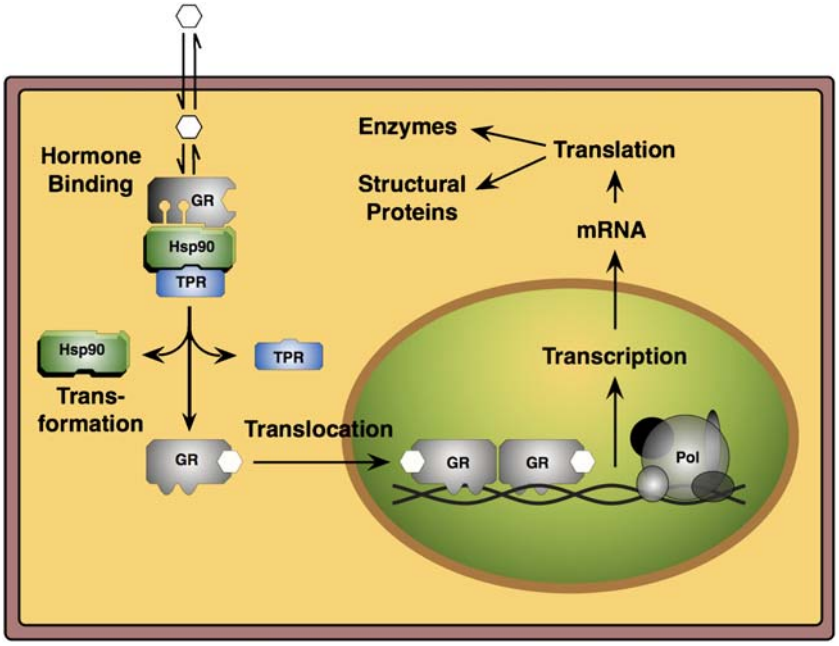

Fig. 2 Classical model of glucocorticoid receptor signaling

consequences of GR action occur over hours, some effects appear within minutes. These non-genomic actions have been shown to involve kinases, phosphatases, and G-protein coupled receptors [20] although the physiological relevance of such nongenomic action of GR remains to be definitively established.

4 Glucocorticoid receptor knockout studies

Transgenic animals have become increasingly essential in identifying the function of specific genes and proteins. In efforts to more clearly define the control GR imparts on physiology, two critical transgenic mouse models have been developed [21]. Knockout of the GR gene in all tissues exerts minimal effects on embryonic development, but results in perinatal lethality as a result of atelectasis of the lungs. Furthermore, alterations of gluconeogenic enzymes in the liver and of the HPA axis can be observed perinatally [15]. A second GR mutation, GR^{dim}, uncovers an essential duality of GR function. These transgenic mice carry a single point mutation, A458T, in one zinc finger of the receptor. This mutation abolishes the ability of receptor to homodimerize and bind to DNA. Therefore, transactivation and transrepression that require direct DNA binding of dimeric GR are inactive, while transrepression involving the tethering of monomeric GR to DNA-bound transcription factors remains intact. Surprisingly, these GR^{dim} mice are viable with no atelectatic lung phenotypes while many anti-inflammatory actions of the receptor are unaffected [22]. Thus, homodimer GR-DNA binding is not essential for survival [23].

5 Molecular aspects of GR function

5.1 Mechanisms and regulation of GR nuclear import and export

The unliganded GR is a multimeric complex consisting of the receptor polypeptide, dimer of HSP90, a molecule of p23, and one tetratricopeptide repeat (TPR) protein (Fig. 3). The HSP90 dimer binds directly to the receptor ligand binding domain [24]. This association is necessary for

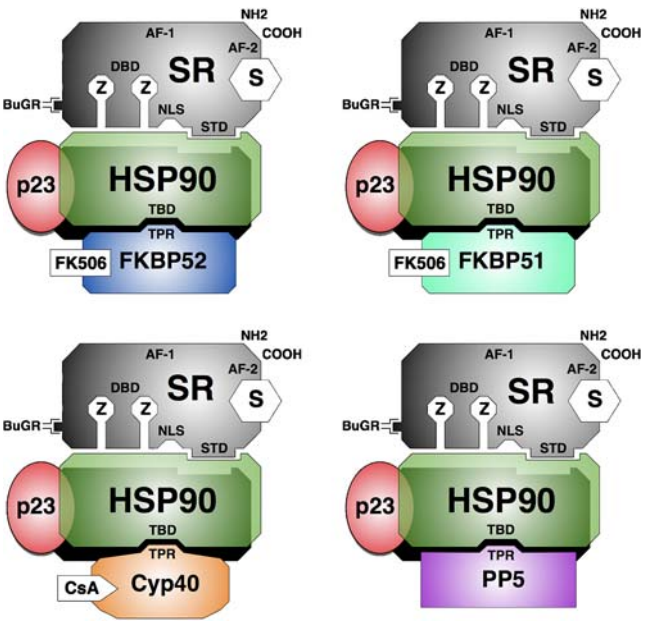

Fig. 3 Four distinct steroid receptor complexes

hormone binding as well as translocation of the receptor [25, 26]. The p23 molecule is involved in stabilizing the hormone binding conformation and binds exclusively to ATP-bound HSP90 [27, 28]. The four main receptor associated TPR proteins are as follows: FKBP52, FKBP51, Cyp40, and PP5 and link with the receptor through the HSP90 dimer. Depending on the state of the receptor (i.e. hormone free vs. hormone bound) the associated TPR protein can differ [29]. In addition, the associated TPR protein is known to vary by receptor type. What remains largely unknown is the role these TPR proteins play in the regulation of steroid receptor activity. Although attachment of TPR proteins to the heteromeric complex is not necessary for receptor function, they modulate receptor activity [30]. The organization of the unliganded receptor and these chaperone proteins constitutes a mature receptor complex that is receptive to hormones [31]. In general, ligand binding activates the receptor, stimulating the initial complex to dissociate.

Distinct signal sequences within the GR are responsible for its transit either into or out of the nucleus. Specifically, two nuclear import signal sequences (NLSs) have been mapped within the GR (i.e. NL1 within the DBD and NL2 within the LBD; [32]) while one nuclear export signal sequence (NES) has been identified within the receptor DBD [33]. NL1 of GR resembles a prototypical NLS in its basic amino acid character and functions when isolated as a hormone-independent NLS [32]. Furthermore, NL1 serves as a recognition site for a number of nuclear import receptors such as importin α2, importin 7 and importin 8 [9, 34]. The precise amino acid determinants of NL2 have not been defined but this NLS has been found to interact with importin 7 and importin 8 [34]. Nuclear export of GR is driven by its interaction with calcium-binding protein calreticulin [33].

While many mechanisms have been found to account for glucocorticoid resistance *in vitro* and *in vivo* [35], in some instances disruptions in GR nuclear import was associated with cellular resistance to glucocorticoids. For example, GR nuclear import is disrupted in a subset of patients with steroid-resistant or steroid-dependent asthma [36]. In addition, impaired nuclear import of GR is one of the mechanisms that accounts for the resistance of some leukemic cells to glucocorticoid-induced apoptosis [37]. Since reductions in GR nuclear import and subsequent glucocorticoid resistance may be influenced by activation of specific signaling pathways and not genetic alterations in the receptor [36], potential targets could exist for therapeutic intervention to reduce GR resistance. For example, phosphorylation of GR following IL-2 and IL-4 activation of the p38 mitogen-activated protein kinase (MAPK) reduces GR nuclear import and can account for glucocorticoid resistance [36].

The regulation of GR nuclear export also appears to be integrated with numerous signaling pathways *in vitro*. For example, phosphorylation of human GR in cultured cells by the MAPK family member c-Jun N-terminal kinase (JNK) at a specific serine residue (i.e. Ser-226) enhances its nuclear export and limits the transactivation properties of the receptor [38]. Other MAPK family members have been implicated in the regulation of steroid receptor nuclear export *in vitro* [39], but the relevance of these results to the regulation of GR function in physiological or pathophysiological contexts has not been established. It is also not known whether the regulation of GR nuclear export by calcium observed *in vitro* is reflective of calcium's effects on nuclear GR *in vivo* [40].

The nucleocytoplasmic shuttling of GR provides a mechanism for the receptor to "sense" cellular hormone status and be exposed to dynamic fluctuations in other signaling pathways both in the cytoplasm and the nucleus. As mentioned above, individual MAPKs may exert a direct effect on the trafficking of GR, and therefore, receptor availability to nuclear or cytoplasmic targets could be influenced by signaling events that are superimposed upon glucocorticoid stimulation. However, nucleocytoplasmic shuttling may also impact the efficacy of GR signaling in the nucleus by altering receptor turnover. When GR nuclear export was enhanced in cultured cells through the linking of a heterologous NES onto the receptor, hormone-dependent downregulation of GR was enhanced [41].

5.2 TPR proteins

The accessory proteins in complex with the steroid receptor-HSP90-p23 share a specific structure: TPR motifs. These motifs, a degenerative sequence of 34 amino acids, facilitate protein–protein interactions and were first discovered as an interactive element of cell division in yeast [42]. A vast number of TPR proteins have since been identified and are found to be involved in a variety of biological processes, i.e., cell cycle control, transcriptional events, splicing, protein transport, phosphate turnover, and protein folding [43].

The first TPR protein discovered to associate with steroid receptors was the immunophilin FKBP52 by Faber et al. in 1984 [44]. Since then two additional immunophilins and one phosphatase have been determined to associate with the mature steroid receptor complex: FKBP51, Cyp40, and PP5, respectively. Of the four TPR proteins that interact with steroid receptors, three are immunophilins (FKBP52, FKBP51, Cyp40). By definition immunophilins are proteins that bind immunosuppressive drugs. FKBP52, FKBP51 and Cyp40 arise from two distinct classes of immunophilins; cyclophilins and FKBPs. Cyclophilins bind cyclosporins whereas FKBPs bind the immunosuppressant macrolide FK506.

Cyclosporin A was first isolated from the fungi *Tolypocladium inflatum* in 1976 and was developed for treatment in transplant surgeries, more specifically kidney and liver allograft rejections [45]. Ten years later FK506 was discovered and is also used with liver and kidney transplants [46]. While FK506 and CsA are structurally different, their modes of action and target proteins are similar. Both inhibit Ca^{2+}-dependent signal transduction pathways with an end result of decreasing T-cell regulated immunity [47]. The immunophilin-ligand complex connects with a phosphatase known as calcineurin (CN) [48]. This action disables CN from dephosphorylating the transcription factor NFAT (nuclear factor of activated T-cells); thereby, prohibiting the nuclear translocation of NFAT and subsequent decrease of cytokines [49].

The TPR domains create an electrostatic interaction with the C-terminal EEVD sequence of Hsp90 allowing for only one TPR protein to interact with the steroid receptor complex [50]. Following identification of these proteins, research has focused on elucidating the role each plays in steroid receptor activity. To date, it has been reported these TPR proteins can enhance or attenuate steroid receptor activity by affecting hormone binding affinity and translocation [51, 52]. Several actions of these TPR proteins appear to be redundant in terms of receptor function. For example, FKBP52 or PP5, when complexed with GR increases its ability to bind hormone [53]. It still remains a mystery as to whether there is a singular specific role for each TPR protein and whether TPR proteins affect each receptor in the same manner.

Pratt and others have investigated the roles that FKBP52 and FKBP51 have in signaling protein movement. The cytoplasmic portion of FKBP52 colocalizes with microtubules. Pratt et al. demonstrated that a specific sequence on FKBP52 was responsible for nuclear translocation. Subsequently, it was determined the PPIase domain of FKBP52 was responsible for its interface with dynein [54]. To elaborate on the essential role of the PPIase domain, a fragment of the PPIase domain competed for the interaction of FKBP52 with dynein and inhibited translocation of the receptor [55]. Following addition of hormone, there is an immunophilin swap of FKBP51 for FKBP52 with the concomitant recruitment of dynein.

New World squirrel monkeys have 50–100 times greater free cortisol levels than humans, yet have a normal hypothalamic–pituitary–adrenal axis, do not display signs of cortisol excess, and have no significant functional variations in their GR gene [56]. The GR in squirrel monkeys exhibits lower hormone binding affinity, impaired translocation, decreased transactivation activity at submaximal hormone concentrations when compared to its human cognate [57, 58]. This glucocorticoid resistance has also been attributed to increased circulating levels of FKBP51; squirrel monkey lymphocytes have a 13-fold higher level of FKBP51 than human lymphocytes [59]. Altering the levels of available FKBP51 via FK506 relieved the inhibitory effect of FKBP51. Thus, an inhibitory role on GR action has been assigned to FKBP51 from studies involving glucocorticoid resistant New World squirrel monkeys [58].

5.3 Steroid receptor coactivators

Steroid receptor coactivators enhance steroid receptor-dependent gene expression through a variety of mechanisms [60]. Coactivator complexes are assembled onto receptor-bound promoters and stimulate steroid receptor-mediated transcription either through direct interactions with the basal transcription machinery or by inducing local chromatin remodeling, including histone acetylation or methylation [60–65]. Some coactivators possess enzymatic activity such as histone acetyltransferase and methyltransferase activities that post-translationally modify histone proteins to affect chromatin structure, while others that lack such activities function to recruit chromatin modifying enzymes to active promoters.

Most coactivators interact with the ligand binding domain of a variety of steroid receptors including GR and AR via nuclear receptor boxes such as LXXLL motifs [66, 67]. However, differences in binding affinities between steroid receptors and their coactivators may play a role in determining the specificity of hormonal responses. Although both AR and GR interact with similar coactivators, it is postulated that unique coactivator complexes may be responsible for specific cellular and gene responses to each hormone.

While many coactivators are likely to be redundant in their effects on steroid receptor function in cultured cell lines, recent *in vivo* studies suggest that different physiological outcomes may result from variations in coactivator expression [64, 65, 67]. For example, TIF-2 and RAC3 null mice display reduced reproductive capability [68, 69]. TIF-2 null male mice have defective spermiogenesis and display testicular degeneration, whereas females display poor placental development, resulting in embryonic growth retardation [69]. RAC3 −/− mice have decreased mammary gland growth, ovulatory capacity, and litter size [68]. Alternatively, whereas SRC-1 null mice are fertile, they exhibit partial hormone resistance [70]. Specifically, SRC-1 −/− mice have decreased growth and development of the uterus, mammary gland, prostate, and testes in response to steroid hormones [70].

Recently, many coactivators have been identified and classified into sub-families consisting of similar members. For example, the p160 family of coactivators, designated steroid receptor coactivators (SRCs) consists of SRC-1 (or

NcoA1), SRC-2 (or TIF-2, GRIP1), and SRC-3 (or p/CIP, RAC3, ACTR, or AIB1). These coactivators share conserved sequence regions termed nuclear receptor interaction domains that permit interactions with a broad range of steroid receptors. Other coactivators that are distinct from the p160 family are components of large complexes, such as the vitamin D interacting proteins (DRIPs) and thyroid receptor associated proteins (TRAPs) [60, 61]. Finally, many other proteins have been identified as androgen receptor activators that may utilize unique mechanisms to impact receptor transactivation that remain largely undefined [62, 63]. For example, ARA70, an AR-specific coactivator, may play a role in uncovering the agonist activity of certain anti-androgens to activate AR activity [71]. Furthermore, a LIM domain containing protein that associates with focal adhesions, hydrogen peroxide-inducible clone-5 (Hic-5/ARA55) was identified as a nuclear receptor coactivator [72, 73].

In recent years, numerous steroid receptor-interacting proteins have been identified that modify chromatin, thereby influencing steroid receptor-mediated gene expression. Because assays using reporter gene analysis to determine steroid receptor activity revealed that overexpression of most coactivators enhanced the activity of many steroid receptors, coactivator specificity was questioned. Hence, it was postulated that coactivators function in a cell type or promoter-specific manner. Using the MMTV promoter, Li et al. isolated a specific coactivator complex that associated with PR that differed to the coactivator complex that bound to GR [74]. These results indicate that combinations of coactivators rather than individual coactivators may be responsible for promoter or hormone-specific gene expression. Furthermore, coactivator knock out analysis provided evidence that at least in part, coactivator function is not totally redundant. For example, TIF-2 and RAC3 null mice display reduced reproductive capability while SRC-1 null mice exhibit partial hormone resistance [69, 70].

5.4 Nuclear receptor corepressors

Along with coactivators, another set of steroid receptor interacting proteins has recently been identified termed corepressors, including both nuclear receptor corepressor (NCoR) and silencing mediator or retinoid and thyroid receptors (SMRT) [75, 76]. Corepressors repress steroid receptor-mediated gene expression in the absence of ligand or presence of antagonist by interacting with histone deacetylases (HDACs) that in turn modify chromatin by removing acetyl groups on histone tails thereby promoting a closed chromatin structure, repressing transcription [32].

Individually, coactivators induce and corepressors repress steroid receptor-mediated gene expression. However, most cells express a combination of both corepressors and coactivators that also interact with each other. For example, RAC3 interacts with NCoR, modifying thyroid hormone receptor (TR) regulated transcription [77]. An equilibrium model hypothesizes that it is not the absolute amount of coregulator expression but the ratio of corepressors versus coactivators that determines the extent of steroid receptor-mediated gene expression [78]. For example, overexpression of SMRT antagonizes TIF-2 coactivation of GR-mediated gene expression [79]. Because coactivators and corepressors, collectively referred to as coregulators, alter steroid receptor-mediated gene expression in endocrine target tissues, their activity and expression along with various steroid receptors, including AR and ER, in prostate as well as breast cancer has been an area of intense investigation.

5.5 Hic-5, a novel GR coactivator

Along with coactivators that modify chromatin, other coactivators such as Hic-5/ARA55 have been discovered whose mechanism of action is largely unknown. Because Hic-5/ARA55 does not possess a catalytic domain that is responsible for its coactivation properties, its mechanism of coregulator function has remained undefined. However, it may serve as an adaptor molecule, either recruiting or stabilizing promoter-specific protein complexes. LIM proteins are well recognized for their roles as molecular adaptors, functioning in stabilizing higher order protein complexes at either focal adhesion complexes or promoter sequences. siRNA ablation experiments establish that Hic-5/ARA55 is required for the stable association of p300 and TIF-2 with the MMTV promoter. Thus, Hic-5/ARA55 may stabilize select protein complex formation at GR-responsive promoters by serving as an adaptor molecule.

Not only does Hic-5/ARA55 interact with various coactivator complexes, but it also associates with NCoR corepressor complexes in the absence of hormone at steroid receptor-responsive promoters. This suggests that Hic-5/ARA55 is capable of interacting with other coregulators directly not necessarily via steroid receptors. Furthermore, because Hic-5/ARA55 is present on GR-responsive promoters in the absence and presence of glucocorticoids, it may function in coordinating corepressor release and coactivator recruitment upon glucocorticoid stimulation.

Recently, transducin β-like 1 (TBL1), an adaptor-like protein, has been reported to mediate the exchange of corepressors for coactivators on steroid receptor-responsive promoters in response to ligand [80]. TBL1 was initially isolated as part of the corepressor complex [81]. ChIP analysis of steroid receptor target promoters revealed prolonged TBL1 promoter association in the presence of

ligand [80]. Furthermore, TBL1 recruited proteosome machinery to steroid receptor target promoters, leading to degradation of the corepressor complex followed by association of the coactivator complex [80]. Although the possible interaction of Hic-5/ARA55 with components of the proteosome machinery has not been analyzed, it may provide a mechanism by which Hic-5/ARA55 interacts with both corepressors and coactivators, culminating in enhanced steroid receptor-mediated gene expression.

6 Regulation of GR protein turnover

Hormone-dependent downregulation of GR is a feature associated with chronic hormone treatment in most cells in culture and tissues *in vivo* [82]. Human leukemic cells are the most well studied exception to this property as prolonged hormone treatment leads to increased GR levels [83]. Interestingly, this auto-induction of GR in leukemic cells appears to be required for glucocorticoid-induced apoptosis [83]. While transcriptional and posttranscriptional mechanisms also contribute to reduced GR protein expression in cells chronically exposed to glucocorticoids [84], we will focus on enhanced protein degradation for our discussions of hormone-dependent GR downregulation.

Like all steroid receptors, GR is primarily degraded via the ubiquitin/proteasome-dependent protein degradation pathway (UPP) [85]. This pathway has been reviewed extensively and will only be briefly described herein [86]. Proteins that are targeted to the proteasome for degradation must be covalently tagged with multiple ubiquitin moieties [87]. The addition of ubiquitin, a 76 amino acid peptide, to target proteins is exquisitely regulated by the sequential action of an ubiquitin activating enzyme (E1), ubiquitin conjugating enzyme (E2) and ubiquitin ligase (E3) [87]. The specificity for protein ubiquitylation lies mainly with the E3 ligases, which are the most diverse components of the ubiquitin modification pathway [87]. The E3 ligase Hdm2 may be particularly relevant to hormone-induced downregulation of GR in cultured human umbilical vein endothelial cells exposed to DNA damaging agents or hypoxia [88]. Furthermore, in human breast cancer cell lines, the ability of estrogen to trigger GR degradation is due to the estrogen-dependent induction of Hdm2 [89]. Carboxyl-terminal hsp70 interacting protein (CHIP) has also been identified as an E3 ligase for GR that promotes both hormone-independent and hormone-dependent degradation of the receptor [90, 91].

While it seems likely that different E3 ligases will be responsible for promoting UPP-driven degradation of GR, it is unclear whether physiological regulation of specific E3 ligases contributes to the steady state GR levels either under basal or hormone stimulated conditions. In a cultured mouse hippocampal cell line, the inability of hormone treatment to trigger GR downregulation can be restored upon overexpression of the CHIP E3 ligase [91]. These *in vitro* experiments suggest that the apparent lack of GR downregulation observed in rat fetal neurons *in vivo* and in primary culture could be due to reduced expression or activity of specific E3 ligases (e.g. CHIP) that act on the receptor in neurons [92, 93].

7 Conclusion

The principal mediator of the physiological actions of glucocorticoids, the GR, is now a well recognized drug target whose modulation is important for the management of many diseases associated with an inflammatory response. Clearly as molecular details are uncovered regarding the mechanisms of gene-specific GR transcriptional regulation, there is hope for the development of specific pharmaceutical manipulation of the GR gene regulatory pathway that would have a high therapeutic index and limit the serious side effects often associated with systemic glucocorticoid treatment.

References

1. Jensen EV. On the mechanism of estrogen action. Perspect Biol Med 1962;6:47–59.
2. Ward E, Slocumb CH, Polley HF, Kendall EC, Hench PS. Clinical effects of cortisone administered orally to 100 patients with rheumatoid arthritis. Ann Rheum Dis 1951;10:477–84.
3. Munck A, Brinck-Johnsen T. Specific and nonspecific physicochemical interactions of glucocorticoids and related steroids with rat thymus cells *in vitro*. J Biol Chem 1968;243:5556–65.
4. Hollenberg SM, Weinberger C, Ong ES, Cerelli G, Oro A, Lebo R, Thompson EB, Rosenfeld MG, Evans RM. Primary structure and expression of a functional human glucocorticoid receptor cDNA. Nature 1985;318:635–41.
5. McEwan IJ, Wright AP, Dahlman-Wright K, Carlstedt-Duke J, Gustafsson JA. Direct interaction of the tau 1 transactivation domain of the human glucocorticoid receptor with the basal transcriptional machinery. Mol Cell Biol 1993;13:399–407.
6. Dahlman-Wright K, Baumann H, McEwan IJ, Almlof T, Wright AP, Gustafsson JA, Hard T. Structural characterization of a minimal functional transactivation domain from the human glucocorticoid receptor. Proc Natl Acad Sci U S A 1995;92:1699–703.
7. Freedman LP, Luisi BF. On the mechanism of DNA binding by nuclear hormone receptors: a structural and functional perspective. J Cell Biochem 1993;51:140–150.
8. Tsai MJ, O'Malley B. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annu Rev Biochem 1994;63:451–86.
9. Savory JG, Hsu B, Laquian IR, Giffin W, Reich T, Hache RJ, Lefebvre YA. Discrimination between NL1- and NL2-mediated nuclear localization of the glucocorticoid receptor. Mol Cell Biol 1999;19:1025–37.

10. MacGregor J, Jordan V. Basic guide to the mechanisms of antiestrogen action. Pharmacol Rev 1998;50:151–96.
11. Beato M, Klug J. Steroid hormone receptors: an update.. Hum Reprod Update 2000;6:225–36.
12. Galigniana MD, Scruggs JL, Herrington J, Welsh MJ, Carter-Su C, Housley PR, Pratt WB. Heat shock protein 90-dependent (geldanamycin-inhibited) movement of the glucocorticoid receptor through the cytoplasm to the nucleus requires intact cytoskeleton. Mol Endocrinol 1998;12:1903–13.
13. Bamberger C, Schulte H, Chrousos G. Molecular determinants of glucocorticoid receptor function and tissue sensitivity to glucocorticoids. Endocr Rev 1996;17:245–61.
14. Newton R. Molecular mechanisms of glucocorticoid action: what is important. Thorax 2000;55:603–13.
15. Cole T, Blendy J, Monaghan A, Schmid W, Aguzzi A, Schutz G. Molecular genetic analysis of glucocorticoid signaling during mouse development. Steroids 1995;60:93–6.
16. Beato M. Gene regulation by steroid hormones. Cell 1989;56: 335–44.
17. Drouin J, Sun YL, Chamberland M, Gauthier Y, De Lean A, Nemer M, Schmidt TJ. Novel glucocorticoid receptor complex with DNA element of the hormone-repressed POMC gene. Embo J 1993;12:145–56.
18. Adcock I, Ito K, Barnes P. Glucocorticoids: effects on gene transcription. Proc Am Thorac Soc 2004;1:247–54.
19. Yang-Yen H-F, Chambard J-C, Sun Y-L, Smeal T, Schmidt TJ, Drouin J, Karin M. Transcriptional interference between c-Jun and the glucocorticoid receptor: mutual inhibition of DNA-binding due to direct protein–protein interaction. Cell 1990;62:1205–15.
20. Hayashi R, Wada H, Ito K, Adcock I. Effects of glucocorticoids on gene transcription. Eur J Pharmacol 2004;500:51–62.
21. Wintermantel T, Berger S, Greiner E, Schutz G. Genetic dissection of corticosteroid receptor function in mice. Horm Metab Res 2004;36:387–91.
22. Reichardt H, Tuckermann J, Gottlicher M, Vujic M, Weih F, Angel P, Herrlich P, Schutz G. Repression of inflammatory responses in the absence of DNA binding by the glucocorticoid receptor. EMBO J 2001;20:7168–73.
23. Reichardt HM, Kaestner KH, Tuckermann J, Kretz O, Wessely O, Bock R, Gass P, Schmid W, Herrlich P, Angel P, Schutz G. DNA binding of the glucocorticoid receptor is not essential for survival. Cell 1998;93:531–41.
24. Simons SJ, Sistare F, Chakraborti P. Steroid binding activity is retained in a 16-kDa fragment of the steroid binding domain of rat glucocorticoid receptors. J Biol Chem 1989;264:14493–7.
25. Bresnick EH, Dalman FC, Sanchez ER, Pratt WB. Evidence that the 90-kDa heat shock protein is necessary for the steroid binding conformation of the L cell glucocorticoid receptor. J Biol Chem 1989;264:4992–7.
26. Czar MJ, Galigniana MD, Silverstein AM, Pratt WB. Geldanamycin, a heat shock protein 90-binding benzoquinone ansamycin, inhibits steroid-dependent translocation of the glucocorticoid receptor from the cytoplasm to the nucleus. Biochemistry 1997;36:7776–85.
27. Cheung J, Smith D. Molecular chaperone interactions with steroid receptors: an update. Mol Endocrinol 2000;14:939–46.
28. Johnson JL, Toft DO. Binding of p23 and hsp90 during assembly with the progesterone receptor. Mol Endocrinol 1995;9:670–768.
29. Davies T, Ning Y, Sanchez E. A new first step in activation of steroid receptors: hormone-induced switching of FKBP51 and FKBP52 immunophilins. J Biol Chem 2002;277:4597–600.
30. Pratt W, Toft D. Steroid receptor interactions with heat shock protein and immunophilin chaperones. Endocr Rev 1997;18: 306–60.
31. Smith D. Tetratricopeptide repeat cochaperones in steroid receptor complexes. Cell Stress Chaperones 2004;9:109–21.
32. Glass CK, Rosenfeld MG. The coregulator exchange in transcriptional functions of nuclear receptors. Genes Dev 2000;14:121–41.
33. Holaska JM, Black BE, Love DC, Hanover JA, Leszyk J, Paschal BM. Calreticulin is a receptor for nuclear export. J Cell Biol 2001;152:127–40.
34. Freedman ND, Yamamoto KR. Importin 7 and importin alpha/importin beta are nuclear import receptors for the glucocorticoid receptor. Mol Biol Cell 2004;15:2276–86.
35. Schaaf MJ, Cidlowski JA. Molecular mechanisms of glucocorticoid action and resistance. J Steroid Biochem Mol Biol 2002;83:37–48.
36. Irusen E, Matthews JG, Takahashi A, Barnes PJ, Chung KF, Adcock IM. p38 Mitogen-activated protein kinase-induced glucocorticoid receptor phosphorylation reduces its activity: role in steroid-insensitive asthma. J Allergy Clin Immunol 2002; 109:649–57.
37. Yamamoto KR, Stampfer MR, Tomkins GM. Receptors from glucocorticoid-sensitive lymphoma cells and two classes of insensitive clones: physical and DNA-binding properties. Proc Natl Acad Sci U S A 1974;71:3901–5.
38. Itoh M, Adachi M, Yasui H, Takekawa M, Tanaka H, Imai K. Nuclear export of glucocorticoid receptor is enhanced by c-Jun N-terminal kinase-mediated phosphorylation. Mol Endocrinol 2002;16:2382–92.
39. Lee H, Bai W. Regulation of estrogen receptor nuclear export by ligand-induced and p38-mediated receptor phosphorylation. Mol Cell Biol 2002;22:5835–45.
40. Holaska JM, Black BE, Rastinejad F, Paschal BM. Ca^{2+}-dependent nuclear export mediated by calreticulin. Mol Cell Biol 2002;22:6286–97.
41. Liu J, DeFranco DB. Protracted nuclear export of glucocorticoid receptor limits its turnover and does not require the exportin 1/CRM1-directed nuclear export pathway. Mol Endocrinol 2000;14:40–51.
42. Sikorski RS, Boguski MS, Goebl M, Hieter P. A repeating amino acid motif in CDC23 defines a family of proteins and a new relationship among genes required for mitosis and RNA synthesis. Cell 1990;60:307–17.
43. Blatch G, Lassle M. The tetratricopeptide repeat: a structural motif mediating protein–protein interactions. Bioessays 1999;21:932–9.
44. Nakao K, Myers JE, Faber LE. Development of a monoclonal antibody to the rabbit 8.5S uterin progestin receptor. Can J Biochem Cell Biol 1984;63:33–40.
45. Borel J. Comparative study of *in vitro* and *in vivo* drug effects on cell-mediated cytotoxicity. Immunology 1976;31:631–41.
46. Fung J, Thomson A, Pinna A, Selby R, Starzl T. State of immunosuppressive agents in organ transplantation. Transplant Proc 1992;24:2372–4.
47. Liu J, Albers M, Wandless T, Luan S, Alberg D, Belshaw P, Cohen P, MacKintosh C, Klee C, Schreiber S. Inhibition of T cell signaling by immunophilin-ligand complexes correlates with loss of calcineurin phosphatase activity. Biochemistry 1992;31:3896–901.
48. Gkika D, Topala C, Hoenderop J, Bindels R. The immunophilin FKBP52 inhibits the activity of the epithelial Ca^{2+} channel TRPV5. Am J Physiol Renal Physiol 2006;290:F1253–9.
49. McCaffrey P, Luo C, Kerppola T, Jain J, Badalian T, Ho A, Burgeon E, Lane W, Lambert J, Curran T, et al. Isolation of the cyclosporin-sensitive T cell transcription factor NFATp. Science 1993;262:750–4.
50. Scheufler C, Brinker A, Bourenkov G, Pegoraro S, Moroder L, Bartunik H, Hartl FU, Moarefi I. Structure of TPR domain-peptide complexes: critical elements in the assembly of the Hsp70–Hsp90 multichaperone machine. Cell 2000;101:199–210.
51. Smith DF. Tetratricopeptide repeat cochaperones in steroid receptor complexes. Cell Stress Chaperones 2004;9:109–21.

52. Davies TH, Ning YM, Sanchez ER. Differential control of glucocorticoid receptor hormone-binding function by tetratricopeptide repeat (TPR) proteins and the immunosuppressive ligand FK506. Biochemistry 2005;44:2030–8.
53. Davies T, Sanchez E. FKBP52. Int J Biochem Cell Biol 2005;37:42–7.
54. Silverstein AM, Galigniana MD, Kanelakis KC, Radanyi C, Renoir JM, Pratt WB. Different regions of the immunophilin FKBP52 determine its association with the glucocorticoid receptor, hsp90, and cytoplasmic dynein. J Biol Chem 1999; 274:36980–6.
55. Galigniana M, Radanyi C, Renoir J, Housley P, Pratt W. Evidence that the peptidylprolyl isomerase domain of the hsp90-binding immunophilin FKBP52 is involved in both dynein interaction and glucocorticoid receptor movement to the nucleus. J Biol Chem 2001;276:14884–9.
56. Chrousos G, Renquist D, Brandon D, Eil C, Pugeat M, Vigersky R, Cutler GJ, Loriaux D, Lipsett M. Glucocorticoid hormone resistance during primate evolution: receptor-mediated mechanisms. Proc Natl Acad Sci U S A 1982;79:2036–40.
57. Her S, Patel P, Schatzberg A, Lyons D. Mutations in squirrel monkey glucocorticoid receptor impair nuclear translocation. J Steroid Biochem Mol Biol 2005;94:319–26.
58. Westberry J, Sadosky P, Hubler T, Gross K, Scammell J. Glucocorticoid resistance in squirrel monkeys results from a combination of a transcriptionally incompetent glucocorticoid receptor and overexpression of the glucocorticoid receptor cochaperone FKBP51. J Steroid Biochem Mol Biol 2006;100: 34–41.
59. Reynolds PD, Ruan Y, Smith DF, Scammell JG. Glucocorticoid resistance in the squirrel monkey is associated with overexpression of the immunophilin FKBP51. J Clin Endocrinol Metab 1999;84:663–9.
60. Onate SA, Tsai SY, Tsai MJ, O'Malley BW. Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. Science 1995;270:1354–7.
61. McKenna NJ, Lanz RB, O'Malley BW. Nuclear receptor coregulators: cellular and molecular biology. Endocr Rev 1999;20:321–44.
62. Bannister AJ, Kouzarides T. The CBP co-activator is a histone acetyltransferase. Nature 1996;384:641–3.
63. Ogryzko VV, Schiltz RL, Russanova V, Howard BH, Nakatani Y. The transcriptional coactivators p300 and CBP are histone acetyltransferases. Cell 1996;87:953–9.
64. Spencer TE, Jenster G, Burcin MM, Allis CD, Zhou J, Mizzen CA, McKenna NJ, Onate SA, Tsai SY, Tsai MJ, O'Malley BW. Steroid receptor coactivator-1 is a histone acetyltransferase. Nature 1997;389:194–8.
65. Swope DL, Mueller CL, Chrivia JC. CREB-binding protein activates transcription through multiple domains. J Biol Chem 1996;271:28138–45.
66. Darimont BD, Wagner RL, Apriletti JW, Stallcup MR, Kushner PJ, Baxter JD, Fletterick RJ, Yamamoto KR. Structure and specificity of nuclear receptor–coactivator interactions. Genes Dev 1998;12:3343–56.
67. Heery DM, Kalkhoven E, Hoare S, Parker MG. A signature motif in transcriptional co-activators mediates binding to nuclear receptors. Nature 1997;387:733–6.
68. Xu J, Liao L, Ning G, Yoshida-Komiya H, Deng C, O'Malley BW. The steroid receptor coactivator SRC-3 (p/CIP/RAC3/AIB1/ACTR/TRAM-1) is required for normal growth, puberty, female reproductive function, and mammary gland development. Proc Natl Acad Sci U S A 2000;97:6379–84.
69. Gehin M, Mark M, Dennefeld C, Dierich A, Gronemeyer H, Chambon P. The function of TIF2/GRIP1 in mouse reproduction is distinct from those of SRC-1 and p/CIP. Mol Cell Biol 2002;22:5923–37.
70. Xu J, Qiu Y, DeMayo FJ, Tsai SY, Tsai MJ, O'Malley BW. Partial hormone resistance in mice with disruption of the steroid receptor coactivator-1 (SRC-1) gene. Science 1998;279:1922–5.
71. Miyamoto H, Yeh S, Wilding G, Chang C. Promotion of agonist activity of antiandrogens by the androgen receptor coactivator, ARA70, in human prostate cancer DU145 cells. Proc Natl Acad Sci U S A 1998;95:7379–84.
72. Fujimoto N, Yeh S, Kang HY, Inui S, Chang HC, Mizokami A, Chang C. Cloning and characterization of androgen receptor coactivator, ARA55, in human prostate. J Biol Chem 1999; 274:8316–21.
73. Yang L, Guerrero J, Hong H, DeFranco DB, Stallcup MR. Interaction of the tau2 transcriptional activation domain of glucocorticoid receptor with a novel steroid receptor coactivator, Hic-5, which localizes to both focal adhesions and the nuclear matrix. Mol Biol Cell 2000;11:2007–18.
74. Cunha GR, Lung B. The possible influence of temporal factors in androgenic responsiveness of urogenital tissue recombinants from wild-type and androgen-insensitive (Tfm) mice. J Exp Zool 1978;205:181–93.
75. Chen JD, Evans RM. A transcriptional co-repressor that interacts with nuclear hormone receptors. Nature 1995;377:454–7.
76. Horlein AJ, Naar AM, Heinzel T, Torchia J, Gloss B, Kurokawa R, Ryan A, Kamei Y, Soderstrom M, Glass CK, et al. Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. Nature 1995;377: 397–404.
77. Li X, Kimbrel EA, Kenan DJ, McDonnell DP. Direct interactions between corepressors and coactivators permit the integration of nuclear receptor-mediated repression and activation. Mol Endocrinol 2002;16:1482–91.
78. Wang Q, Blackford JA Jr., Song LN, Huang Y, Cho S, Simons SS Jr. Equilibrium interactions of corepressors and coactivators with agonist and antagonist complexes of glucocorticoid receptors. Mol Endocrinol 2004;18:1376–95.
79. Szapary D, Huang Y, Simons SS Jr. Opposing effects of corepressor and coactivators in determining the dose–response curve of agonists, and residual agonist activity of antagonists, for glucocorticoid receptor-regulated gene expression. Mol Endocrinol 1999;13:2108–21.
80. Perissi V, Aggarwal A, Glass CK, Rose DW, Rosenfeld MG. A corepressor/coactivator exchange complex required for transcriptional activation by nuclear receptors and other regulated transcription factors. Cell 2004;116:511–26.
81. Li J, Wang J, Nawaz Z, Liu JM, Qin J, Wong J. Both corepressor proteins SMRT and N-CoR exist in large protein complexes containing HDAC3. Embo J 2000;19:4342–50.
82. Bellingham DL, Sar M, Cidlowski JA. Ligand-dependent down-regulation of stably transfected human glucocorticoid receptors is associated with the loss of functional glucocorticoid responsiveness. Mol Endocrinol 1992;6:2090–102.
83. Ramdas J, Liu W, Harmon JM. Glucocorticoid-induced cell death requires autoinduction of glucocorticoid receptor expression in human leukemic T cells. Cancer Res 1999;59:1378–85.
84. Oakley RH, Cidlowski JA. Homologous down regulation of the glucocorticoid receptor: the molecular machinery. Crit Rev Eukaryot Gene Expr 1993;3:63–88.
85. Wallace AD, Cidlowski JA. Proteasome-mediated glucocorticoid receptor degradation restricts transcriptional signaling by glucocorticoids. J Biol Chem 2001;276:42714–21.
86. Voges D, Zwickl P, Baumeister W. The 26S proteasome: a molecular machine designed for controlled proteolysis. Annu Rev Biochem 1999;68:1015–68.
87. Ciechanover A, Orian A, Schwartz AL. Ubiquitin-mediated proteolysis: biological regulation via destruction. Bioessays 2000;22:442–51.

88. Sengupta S, Wasylyk B. Ligand-dependent interaction of the glucocorticoid receptor with p53 enhances their degradation by Hdm2. Genes Dev 2001;15:2367–80.
89. Kinyamu HK, Archer TK. Estrogen receptor-dependent proteasomal degradation of the glucocorticoid receptor is coupled to an increase in mdm2 protein expression. Mol Cell Biol 2003;23:5867–81.
90. Connell P, Ballinger CA, Jiang J, Wu Y, Thompson LJ, Hohfeld J, Patterson C. The co-chaperone CHIP regulates protein triage decisions mediated by heat-shock proteins. Nat Cell Biol 2001;3:93–6.
91. Wang X, DeFranco DB. Alternative effects of the ubiquitin-proteasome pathway on glucocorticoid receptor down-regulation and transactivation are mediated by CHIP, an E3 ligase. Mol Endocrinol 2005;19:1474–82.
92. Wang X, Pongrac JL, DeFranco DB. Glucocorticoid receptors in hippocampal neurons that do not engage proteasomes escape from hormone-dependent down-regulation but maintain transactivation activity. Mol Endocrinol 2002;16:1987–98.
93. Ghosh B, Wood CR, Held GA, Abbott BD, Lau C. Glucocorticoid receptor regulation in the rat embryo: a potential site for developmental toxicity. Toxicol Appl Pharmacol 2000;164:221–9.