Mechanism of action of corticosteroids in inflammatory gene repression

K. De Bosscher, W. Vanden Berghe & Guy Haegeman

Department of Molecular Biology, University of Gent, Ledeganckstraat 35, 9000 Gent (Belgium)

1. Introduction

Inflammation was first described in history by Cornelius Celsus (°30 B.C- †38 A.D.) as a process that embraces “rubor et tumor cum calor et dolor”, i.e. redness and swelling accompanied by heat and pain. Inflammation is the result of a series of enzymatic processes in the body. Cell membrane damage caused by a cut or scratch for example, leads to the activation of phospholipases, which mediate the release of arachidonic acid. This metabolite is further processed by cyclo-oxygenases and lipo-oxygenases to produce the fever-causing prostaglandins, thromboxanes and leukotriens. These fatty acid derivatives have a vasodilatory action, causing a higher blood flow to help attracting inflammatory cytokines and immune cells to the site of inflammation. This explains the accompanying symptoms of redness and swelling in inflamed tissue. The inflammation process is further mediated and controlled by the action of several messenger molecules called cytokines, chemokines and adhesion molecules including TNF-α, IL-1, IL-2, IL-6, MCP-1, IL-8, GM-CSF, ICAM-1 and E-selectin (Barnes & Karin, 1997; Cato & Wade, 1996). These cytokines are produced by (and on their turn activate) different surrounding cell types, such as fibroblasts, endothelial cells (lining blood vessels) and macrophages and neutrophils; white blood cell components travelling through the bloodstream. Upon activation, the latter two cell types attach to the endothelial cell layer by a process called leukocyte rolling and subsequently migrate through the endothelial membrane (called diapedesis) to the place of inflammation. The infection is finally nipped in the bud by the local release of degrading enzymes, such as elastase and cathepsine, oxygen radicals and the action of phagocytosis (‘eating’ remaining debris by immune cells). Signalling by growth factors such as TGF-β promotes cell proliferation and contributes to the process of wound healing.

The dimeric transcription factor NF-κB (mainly p65 and p50) is now recognized as one of the most important regulators of pro-inflammatory gene expression. The activating agents and activation mechanism will be more extensively discussed in chapters 1 and following. Cytokines, chemokines and adhesion

molecules, which are upregulated during an inflammatory insult, contain responsive elements for NF-κB in their promoter region. The action of this transcription factor therefore represents an obvious target for many new anti-inflammatory therapeutic strategies.

2. Glucocorticoid hormones

As early as in the 1930s, the hormone cortisone was isolated from the adrenal glands and its efficacy for treatment of rheumatoid arthritis was empirically demonstrated in patients suffering from this debilitating disease. Due to their immunosuppressive effects, corticosteroids or glucocorticoid hormones (GCs) are used to reduce organ rejection after transplantation and to treat auto-immune diseases, including multiple sclerosis, rheumatoid arthritis and inflammatory bowel disease. Other inflammatory diseases for which GCs make part of the standard treatment are systemic lupus erythematosus (SLE), sarcoidosis, asthma (Barnes & Adcock, 1997) and atopy (Beyaert, 1999). They are also used to treat brain edema, shock conditions, certain types of blood cancer (B- and T-cell lymphoma) (Jehn & Osborne, 1997), as well as conditions involving adrenal cortex insufficiency. GCs inhibit leukocyte migration to sites of inflammation and thus reduce the general symptoms of inflammation (Cato & Wade, 1996). The synthesis and secretion of cortisol, the naturally occurring glucocorticoid in humans, is subject to a negative feedback loop and under tight control by a careful balance between adrenocorticotropin hormone, secreted from the pituitary gland in the brain and corticotropin hormone, secreted from the hypothalamus in a pulsatile and circadian fashion (Balsalobre *et al.*, 2000). GCs exert their functions through binding to the glucocorticoid receptor (GR), a transcription factor that can regulate genes in a positive or negative way. Transcription factors are proteins that are able to bind the promoter regions of their target genes and can modulate the rate of gene transcription. In a resting cell they can either already be in the nucleus bound to DNA and waiting to be activated (e.g. by a phosphorylating signal), or they can reside in the cytoplasm kept in an inactive state before activation allows them to travel inside the nucleus. Just as NF-κB and its set of specific inhibitor molecules IκB, inactive GR is sequestered in the cytoplasm, although not via a specific inhibitor but through its interaction with chaperoning molecules including Hsp90 and Hsp70. Because of their lipophilic nature, ligands for GR (cortisol, corticosterone or the synthetic dexamethasone DEX) can travel through the cell membrane, circumventing the need for membrane receptors to transmit their signal. Ligand binding

induces a conformational change in GR, causing the release of the interacting molecules and exposing its nuclear localisation signal (a stretch of basic residus recognized by importin nuclear transport proteins). Once in the nucleus GR can bind as a homodimer onto the glucocorticoid response element (GRE), an imperfect palindromic recognition sequence GGTACAnnnTGTYCT (Y=T or C), and positively regulate steroid-responsive genes regulating metabolic homeostasis. The GR protein consists of three main protein modules, an N-terminal heavily phosphorylated transactivating domain, a C-terminal dimerisation and ligand binding domain (LBD) and a central DNA-binding domain (DBD) of which eight out of nine cysteins form two tetrahedric structures in which a Zn ion is held. This special structure mediates contact with the DNA; GR is therefore also called a Zinc finger protein. Although these 3 modules can work quasi independently of each other, there is a great overlap in certain functions. For example, transactivation functions are also found in the DBD and LBD and nuclear translocation is not only found back in the LBD but also in the DBD.

GR can also influence gene expression indirectly. A recently recognized important function of activated GR is the inhibition of transcription of several cytokines and chemokines that are relevant in inflammatory diseases. In the last decade it has become evident that one of the main targets for GC-mediated cytokine gene suppression is NF-κB. Recent data**,** derived from elegant studies with dimerisation-defective (and hence transactivation-defective since only a dimeric GR recognizes the GRE consensus sequence) knocked-in GR mutant receptors in mice**,** demonstrated that the anti-inflammatory action of GCs solely arises from the 'negative' cross-talk of GR with NF-κB or AP-1 (Reichardt *et al*., 1998; Reichardt *et al*., 2001). The interaction between different transcription factors resulting in either cooperative enhancement or inhibition of gene expression is referred to as 'cross-talk'. This is an important concept, as cross-talk provides an additional platform of regulation to increase the number of possible gene-specific responses in a cell-specific manner. The long-term use of GCs in patients with chronic inflammatory disorders is, sadly enough, overshadowed by severe metabolic side effects, ascribed to the transactivating function of GR. Endogenous GCs protect the body from stress by regulating blood pressure levels, blood glucose levels, liver glycogen deposition and lipid metabolism. Consequently, a long-term treatment with glucocorticoids can result in diabetes, redistribution of fat and hypertension, but also HPA axis (hypothalamo pituitary adrenal axis) insufficiency, osteoporosis, skin and muscle atrophy, increased

susceptibility to infections, cataract, peptic ulcers and a general retention of water due to a disturbed water household balance (because high levels of GCs can also activate the mineralocorticoid receptor)(Boumpas *et al*., 1993; Karin, 1998). Besides its metabolic actions, GCs also affect brain functions such as behaviour and memory (De Kloet *et al*., 1998); long-term steroid use may therefore also lead to neuropsychiatric conditions. An additional problem is the fact that patients treated with GCs for long periods may develop a resistance towards a steroid-based therapy (Barnes, Greening & Crompton, 1995). For all those reasons, the quest for 'better' anti-inflammatory drugs, separating the beneficial from the detrimental effects (so-called dissociated GCs) is a vigorous one. Progress has been made with the recent development and characterization of 'dissociating' glucocorticoids, which can separate to a certain extent transrepression from transactivation functions of GR (Resche-Rigon & Gronemeyer, 1998; Vanden Berghe *et al*., 1998). In order to improve further therapies for inflammatory disorders, the understanding of the molecular action mechanism of GCs is an absolute prerequisite. By no means a consensus has been reached with regard to the mechanism deployed by GCs in mediating pro-inflammatory gene repression.

Different models aiming to explain this phenomenon have been put forward and will be discussed below.

3. Cytoplasmic models

3.1. Upregulation of IκB-α by glucocorticoids

One way GCs could repress NF-κB-driven gene expression is by sequestration of NF-κB in the cytoplasm. In the resting state, NF-κB activation is tightly controlled by its cytoplasmic inhibitor, IκB-α, which associates with NF-κB and prevents its migration to the nucleus. Two independent research groups proposed that GCs induced IκB-α. This newly made IκB-α would then travel to the nucleus to capture NF-κB from the DNA, ultimately resulting in retention of NF-κB in the cytoplasm and thus inhibition of cytokine gene expression (Auphan *et al*., 1995; Scheinman *et al*., 1995a). This mechanism was demonstrated for monocytes and T cells, but other groups including ours did not find evidence for this mechanism in other cell lines including lung epithelial, fibroblasts and endothelial cells (Adcock *et al*., 1999; Brostjan *et al*., 1996; De Bosscher *et al*., 1997; Hofmann *et al*., 1998). Actually, in many cases GC-mediated repression occurred without a concomitant decrease in the DNA-binding capacity of NF-κB, as determined by gel retardation assays (Brostjan *et al*., 1996; De Bosscher *et al*., 1997; Newton *et al*., 1998).

On top of that, in some cases IκB-α was found to be upregulated upon GC treatment but quite unexpectedly also without an apparent loss or decrease of NF-κB binding (Lezoualc'h *et al*., 1998; Wissink *et al*., 1998). It was recently shown for the ICAM-1 gene by *in vivo* footprinting analysis that GC repression happens without a change in conformation of the protein complex that binds to the NF-κB binding site (Liden *et al*., 2000).

It thus seems that the upregulation of IκB-α by glucocorticoids is a cell-specific phenomenon first observed for monocytes and T-lymphocytes (Auphan *et al*., 1995; Scheinman *et al*., 1995a). However, this observation is not unique or universal to immune cells. For instance, GCs do not upregulate IκB-α in CD4+ cells *in vivo* (Reichardt *et al*., 2001), whereas in cell types, such as PC12, hepatocytes and breast carcinoma cells, GCs do cause an increase in IκB-α protein levels (De Vera *et al*., 1997; Lezoualc'h *et al*., 1998; Ray & Searle, 1997).

An interesting finding proving the point of cell specificity is that in the neuronal cortex of DEX-treated rats no IκB-α was upregulated, whilst in the peripheral cells of the same animal IκB-α levels were found to be enhanced (Unlap & Jope, 1997). Another example reflecting differences between different cell types are exemplified by the following observations. Elevated levels of IκB-α were found in vascular endothelial tissue but not in mononuclear cell infiltrates from GC-treated patients with Crohn's disease. Note that this result also seems to clash with the *in vitro* data, in which mainly T-cells seemed to respond to GCs with a higher IκB-α protein level (Thiele *et al*., 1999). As an IκB-α promoter construct was unresponsive to upregulation by DEX in L929sA cells, the actual mechanism by which GCs enhance IκB-α levels in other cell types is even more puzzling (Vanden Berghe *et al*., 1999b).

Establishing the upregulation of IκB-α by GCs is one thing but the critical question is whether this effect can also be linked to the GC-repression of NF-κB driven genes.

The answer is negative. Following observations have clearly pointed out that IκB-α upregulation by GCs can be uncoupled from their anti-inflammatory cytokine gene-repressive effects. Mutational analysis of GR has shown that its DNA-binding capacity is dispensable for mediating transrepression on NF-κB-driven genes (Caldenhoven *et al*., 1995). This is confirmed *in vivo* by experiments using mice with a knocked-in dimerisation-defective GR $^{dim/dim}$ mutant (A458T), which has lost DNA-binding and gene-activating

properties (Reichardt *et al*., 1998). Vice versa, a GR mutant (S425G) defective in NF-κB targeted gene repression was shown to still be capable of mediating enhanced IκB-α synthesis (Tao, Williams-Skipp & Scheinman, 2001). The repressive effects of GCs further remained apparent in the presence of the protein synthesis inhibitor cycloheximide, presenting evidence that novel protein synthesis is not required to inhibit NF-κB driven gene expression (De Bosscher *et al*., 1997; Wissink *et al*., 1998). Finally, the use of 'dissociated' compounds which lack GR transactivating capacities demonstrated that GR-mediated transcription is not required for the inhibition of p65 transactivation and, reciprocally, GC analogues which lack anti-inflammatory properties *in vivo* could still upregulate IκB-α (Heck *et al*., 1997; Vanden Berghe *et al*., 1999b).

The question remains why in some cell types IκB-α is upregulated by GCs and in other not. One possible explanation could be a functional difference with regard to apoptosis. NF-κB has been reported to have an anti-apoptotic function, as evidenced by the high level of liver apoptosis and subsequent death observed in p65-knockout embryos (Beg *et al*., 1995). Furthermore, NF-κB transcriptional activity has been implicated in cell cycle progression (Hinz *et al*., 1999; Kaltschmidt *et al*., 1999). In contrast, normal T-lymphoid and monocytic cells are sensitive to GC-induced apoptosis, a characteristic of GR used to its advantage in lymphoid cancers. Now, T-cells of GR $^{dim/dim}$ mice were no longer subject to GC-mediated apoptosis, arguing for the need of gene inductive effects by GR to mediate this event. IκB-α induction was found in a GC-induced apoptosis-sensitive cell line, but not in resistant human leukemic T cells (Ramdas & Harmon, 1998). Taken together, in lymphoid cells (which contain a high constitutive level of protective NF-κB) or other cells that have suffered too much damage, the IκB-α upregulation by GCs may ensure a functional apoptotic program to limit systemic immune responses that can otherwise lead to a lethal shock of the whole organism.

3.2. Interference by other signal transduction pathways

A completely different mechanism by which GCs may exert part of their anti-inflammatory effects is the inhibition of signalling pathways that regulate inflammatory processes. One such example is the extracellular regulated kinase ERK-1,2, controlling the release of allergic mediators and induction of pro-inflammatory cytokine gene expression in mast cells. Recently, the mechanism by which glucocorticoids

inhibit ERK kinase activity was unraveled. This involves the increased expression and, crucially, at the same time a diminished proteosomal degradation of MAP kinase phosphatase-1 (Kassel *et al*., 2001). In other cell lines however, such as L929sA mouse fibroblasts, GCs did not inhibit tumor necrosis factor (TNF)-activated ERK activity (De Bosscher, Vanden Berghe & Haegeman, 2001). The lack of blockage of MKP-1 degradation by GCs in these cells, as observed for NIH3T3 mouse fibroblasts (Kassel *et al*., 2001), is probably responsible for the differential outcome. The anti-inflammatory action of glucocorticoids may therefore not only be due to negative regulation by GR, but can also involve positive regulation by this receptor. Other examples of anti-inflammatory proteins upregulated by GCs include secretory leukocyte protease inhibitor, which protects healthy lung tissue from leukocytes activated during airway inflammation (Abbinante-Nissen, Simpson & Leikauf, 1995), β-4-sulfoxide, which has a potent anti-inflammatory action on monocytes and macrophages (Young *et al*., 1999), IL1-receptor antagonist and lipocortin I and II, which are phospholipase inhibitor proteins (Barnes, 1998). Although, lipocortin upregulation by GCs may rather be a tissue or cell-specific effect since GCs did not affect its synthesis in L929sA fibroblasts and could therefore not be held responsible for the protective effects of DEX to TNF-mediated cytotoxicity (Beyaert *et al*., 1990). Along the same line, GC-mediated inhibition of c-Jun N-terminal kinase (JNK) activity leads to inhibition of c-Jun phosphorylation. This helps explaining the repressive action of GR towards the activity of AP-1, another transcription factor involved in pro-inflammatory gene expression (Caelles, Gonzalez Sancho & Muñoz, 1997; De Bosscher *et al*., 2001; Swantek, Cobb & Geppert, 1997). Similar results have been found for the inhibition of p38 MAPK activity by GCs, although this again seems not to be a universal mechanism (De Bosscher *et al*., 2001; Lasa *et al*., 2001). The inhibition of p38 activation requires *de novo* protein synthesis (Lasa *et al*., 2001); in contrast, it was shown that GC-mediated repression of TNF-induced IL-6 mRNA occurred in the presence of cycloheximide, a protein synthesis inhibitor (De Bosscher *et al*., 1997). These differences may reflect subtle regulations by GR to enhance its repressive capacity over a longer period and again emphasize the diversity of regulatory possibilities GR has at hand to modulate cellular signalling events.

Another type of signalling cascade reported to have an effect on NF-κB and/or GR-driven transactivation is the protein kinase A (PKA) pathway. NF-κB-driven transcription is regulated through phosphorylation of RelA by PKA. Phosphorylation of Ser276 in p65 was shown to be essential for complex formation with the

coactivator molecule CBP (cAMP response element binding (CREB)-binding protein) and subsequent stimulation of transactivation (Zhong, Voll & Ghosh, 1998). The catalytic subunit of PKA (PKAc) is also able to potentiate GR-dependent transcription.

Phosphorylation of a serine residu (Ser 276) in the RHD by PKA was further demonstrated to be essential for p65-mediated repression of GR transactivation. Mutation of p65 at this conserved PKA phosphorylation site abolished the potential of p65 to repress GR (Doucas *et al*., 2000). Strikingly, an exclusively cytoplasmic variant of p65 (obtained by deleting the NLS of p65) was still capable of mediating transrepression of a GR-activated mouse mammary tumor virus promoter-driven reporter gene. From this result it was concluded that targeting GC-driven gene expression by p65 occurs in the cytoplasm, involving PKAc-dependent signalling as the molecular interface of this inhibition (Doucas *et al*., 2000)

For the reciprocal mechanism controversy arises. A GR deletion variant (amino acids 589-697 deleted) with a predominant cytoplasmic localization would also still inhibit NF-κB driven gene expression, arguing that a competition for PKAc would mediate mutual cross-coupling in the cytoplasm (Doucas *et al*., 2000). Mapping GR functions has however demonstrated that there is more than one NLS in GR (Beato, 1989; McEwan, Wright & Gustafsson, 1997). It can therefore not be ruled out that a minor proportion of this variant can still travel in and out of the nucleus, using the other NLS, and mediate gene repression in the nucleus.

Furthermore, Ser276 was found not to be a key player for repression of NF-κB activity by GR. In this case, mutation of Ser276 to a Cysteine residu in a Gal4-p65 fusion protein did not affect the ability of GR to block NF-κB-driven transcription (De Bosscher *et al*., 2000b) (the use of Gal4 fusion proteins is discussed in more detail in the section below). It becomes more and more clear that the reciprocal mechanisms of transrepression, i.e. the mutual antagonistic effects of NF-κB and glucocorticoids, do not necessarily use the same molecular mechanisms.

4. Nuclear models

4.1. Direct protein-protein interaction

Direct binding of GR to DNA via a so-called ‘nGRE’ and as such negatively regulating gene expression is quite a rare event. One example of this is the osteocalcin gene. The osteocalcin promoter

region to which GR binds is partially overlapping with the TATA box, occluding the build-up of a functional transcriptional complex (Meyer, Carlstedt Duke & Starr, 1997). In spite of the ability of glucocorticoids to induce gene transcription, the major anti-inflammatory effects of GCs occur through repression of inflammatory and immune genes that are driven by NF-κB or another mitogenic transcription factor, AP-1 (Adcock & Caramori, 2001). Intriguingly, the transrepressive relationship between GR and NF-κB appears to be mutual so it seemed most logic at the time that both proteins would actively hinder each other's transactivating functions by no other means than a direct physical contact. The model of a direct interaction between GR and NF-κB was supported by several research groups for the last 5 years, however, only recently an actual physical interaction with endogenous proteins was demonstrated in A549 lung carcinoma cells (Adcock, Newton & Barnes, 1997). Some research groups have focused on mapping the involved functional domains in transrepression; albeit with sometimes conflicting results. For GR, domain swapping of its modular parts (N-terminal domain, DNA-binding domain (DBD) and ligand binding domain (LBD)) with other members of the nuclear receptor superfamily such as estrogen receptor (ER) and thyroid receptor (TR) demonstrated that the DBD was indispensable, not only for transactivation (as expected) but also for transrepression (Liden *et al*., 1997; Moras & Gronemeyer, 1998; Ray *et al*., 1997). To discern whether the DNA binding function of GR per se was crucial for NF-κB transrepression point mutations in the P-box (=DNA interacting N-terminal Zn finger) were performed. The outcome hereof was that GR binding onto a classical GRE could be disrupted, but the transrepressive properties on NF-κB or AP-1 remained untouched (Caldenhoven *et al*., 1995; Heck *et al*., 1994; Helmberg *et al*., 1995). Another study, however, pointed to the other Zn finger, C-terminally placed, as a crucial determinant for NF-κB transrepression (Liden *et al*., 1997). The reason for this discrepancy remains undetermined but may reside in the nature of the investigated NF-κB response elements (e.g. binding different NF-κB family members) in different cell lines. It is equally possible that a different cofactor context (see further) or GR function-modulating chaperones in different cell lines may be contributing to these discrepancies. Recently, it became apparent that the promoter context may codetermine whether or not a specific nuclear receptor can interfere with NF-κB activity (Amrani, Lazaar & Panettieri, 1999; De Bosscher, Vanden Berghe & Haegeman, 2000a). Replacing the LBD with a non-related inert β-galactosidase moiety (to diminish

unwanted effects of an incorrect folding of deletion variants), did not affect transrepression, arguing for a purely steric role of the ligand binding domain (Oro, Hollenberg & Evans, 1988).

Conversely, also the domains of p65 involved in repression of GR activity have been mapped. Elaborate mutational analysis demonstrated that both the N-terminal Rel homology domain (RHD), containing DNA binding and dimerisation functions, and the C-terminal domain of p65, harbouring transactivation functions, are required for the repression of GR activity. However, *in vitro* a physical interaction was only observed between the RHD of p65 and GR (Scheinman *et al.*, 1995b; Wissink *et al*., 1997).

A greater part of the gathered data points out that the protein-protein interaction between GR and NF-κB is most likely to occur in the nucleus. A first piece of evidence has been referred to above, namely the unchanged footprinting pattern of NF-κB bound to its response element in the GC-repressible ICAM promoter (Liden *et al*., 2000). Secondly, when p65 is fused to a DNA-binding yeast protein Gal4 this fusion protein is completely nuclear and now able to transactivate a GAL4 binding site containing reporter gene. Importantly, GCs can inhibit the transactivation of Gal4-p65 to the same extent as of wild-type p65, arguing that GC repression is a nuclear phenomenon (De Bosscher *et al*., 1997). The transactivating C-terminus of p65 has been shown to contact the general transcription factors TFIIB and TBP (TATA-binding protein) *in vitro* (Schmitz *et al*., 1995); this binding could help stabilizing TFIID interactions to build up a functional promoter initiation complex (PIC) and to start gene transcription. The importance of the promoter context close to the TATA box sequences in mediating transrepression was exemplified by the finding of a NF-κB-driven gene that was no longer responsive to GC-mediated repression (De Bosscher *et al*., 2000b). Finding the sequences that determine this unexpected promoter specificity is an important issue. Another study exploring events around the start site of transcription came up with exciting new evidence that GR mediates repression by interfering with the phosphorylation of a Serine residu in the C-terminal domain of RNA polymerase II, again, without inhibiting assembly of the PIC (Nissen & Yamamoto, 2000). Cofactors are nuclear proteins that are able to form a bridge between transcription factors and the basal transcription machinery, without contacting the DNA themselves (Horwitz *et al*., 1996). They are often associated with an enzymatic activity, either a histone acetylase or a histone deacetylase activity, depending on their function as a coactivator or a corepressor, respectively. Histone acetylation is believed to be important for relaxing chromatin and favouring gene transcription, the opposite

holding true for histone deacetylation (Wolffe & Pruss, 1996; Wolffe, 1997). The abovementioned result postulates the existence of a novel type of corepressor, associated with the LBD of GR, possibly a serine-2-phosphatase or a serine-2 kinase inhibitor (Nissen & Yamamoto, 2000).

A logic question now is whether a cytoplasmic model is in any way reconcilable with a nuclear model. Different findings, dependant on which cell lines are used, have sometimes led to seemingly conflicting results. However, it is quite acceptable that a different constitution in cofactor complexes or a different subset of specific responsive target genes may integrate all the signals coming from the cytoplasm with subtle differences, thus generating a slightly different mechanistic response by GCs. Also, a dosage effect or the duration of the pro-inflammatory insult to the cells may be of importance to the way GCs go about to inhibit the signalling mediated by NF-κB. Besides the transcriptional effects discussed here, important GC effects have also been detected at the posttranscriptional level, such as mRNA destabilization of pro-inflammatory genes (viz. INOS, TNFα, GM-CSF, COX-2) (Chaudhary & Avioli, 1996; Delany, Gabbitas & Canalis, 1995; Lasa *et al*., 2001; Tobler *et al*., 1992). Functional GR must therefore be considered more as context-dependent, multi-targeting effectors, rather than as mediators of repression via one exclusive pathway.

4.2. cofactor models

As mentioned in the paragraph above, cofactor molecules provide an extra layer of transcriptional regulation in the nucleus. Coactivators are generally associated with a HAT (histone acetylase) activity, corepressors are associated with a HDAC (histone deacetylase) activity (Wolffe, wong & Pruss, 1997). The LBD of GR has been shown to interact, in a ligand-dependent way, with coactivators such as CBP/p300, GRIP1 and SRC-1 (Chakravarti *et al*., 1996; Eggert *et al*., 1995; Onate *et al*., 1995). The same coactivators have also been implicated in bridging other transcription factors, such as NF-κB and AP-1 to the basal transcription machinery (Gerritsen *et al*., 1997; Kamei *et al*., 1996; Perkins, 1997; Sheppard *et al*., 1998; Vanden Berghe *et al*., 1999a). Gene repression could therefore result from a competition between transcription factors for limiting amounts of coactivator molecules. A competition between p65 and GR for limiting amounts of CBP or SRC-1 was proposed to account for transrepression of NF-κB-dependent genes (McKay & Cidlowski, 1998; Sheppard *et al*., 1998). It is, however, difficult to understand how this

mechanism would generate a specific transrepression of GC-repressible NF-κB- or AP-1-driven target genes only, since a great number of other transcription factors utilize CBP/p300 or SRC-1 as well for enhancing their transactivation properties. Amongst these are e.g. CREB, ATF-2, MyoD, p53, Tax and STAT-2 (reviewed in (Horwitz *et al*., 1996; Xu, Glass & Rosenfeld, 1999)), although preferences and differences have been noted. So, it has been reported that NF-κB-mediated transactivation requires the presence of CBP, SRC-1 and p/CAF, using mainly the HAT activity of p/CAF, while CREB for instance uses CBP, p/CIP and p/CAF, but not SRC-1 (Korzus *et al*., 1998; Sheppard *et al*., 1999). It may be that a constitution of different coactivator complexes provides a platform for the specific recognition and repression by GCs of certain transcription factor families only. Nevertheless, other experimentations argue against a cofactor squelching model to explain mutual transrepression between NF-κB and GR. Increasing the coactivator levels in the cell by transient overexpression generates a general dose-responsive increase in gene expression levels of NF-κB-driven gene expression. However, upon activating GR the relative repression levels remain unaffected. Furthermore, in case of a competitive mechanism one would expect that the interaction between p65 and CBP would be diminished or completely lost upon interaction with activated GR. This proves not to be the case (De Bosscher *et al*., 2000a). Additional evidence diminishing the role of CBP in transrepression is the fact that the GAL4 transactivating Gal4-p65 Ser276Cys mutant, leading to a defective CBP recruitment and subsequent loss of TNF inducibility, still demonstrated a functional repression by GCs (De Bosscher *et al*., 2000b). For the reciprocal mechanism, p65 mutation in the DNA-binding domain but with the predicted coactivator recruitment domains intact could no longer repress GC-mediated transactivation (Wissink *et al*., 1997). Finally, from the fact that dissociating ligands or GR point mutants can distinguish between transactivation and transrepression it can be deducted that GR is not always associated with the same cofactor surrounding. In fact, a different steric conformation of GR could lie at the basis for this phenomenon, achieving transrepression, when GR adopts a monomeric conformation as opposed to allowing transactivation of target genes, when GR is in a DNA-bound dimeric conformation (Lefstin & Yamamoto, 1998; Reichardt *et al*., 2001). These findings demonstrate an incompatibility with a general cofactor competiton model.

It must be noted that all the abovementioned experimental approaches depending on overexpression and microinjection overload the cell with transcriptional components, disregarding the influence of nuclear

architecture and specific nuclear matrix targeting. This latter phenomenon is a concept that has recently gained importance. Territorial subdivision of transcription factor complexes in the nucleus (Doucas *et al*., 1999; Stenoien *et al*., 2000; Stewart & Crabtree, 2000) may explain how cofactors only target a certain gene in a designated compartment in the nucleus whilst leaving the same factors associated with different genes in other compartments intact (Francastel *et al*., 2000; Hager *et al*., 2000; Lemon & Tjian, 2000). A specific nuclear matrix targeting signal has been described to include parts of the DBD and transactivation domains of GR (DeFranco & Guerrero, 2000).

Some members of the nuclear hormone receptor superfamily, such as Retinoic/Retinoid X Acid Receptors RAR/RXR and Thyroid Receptors (TR) are already bound to DNA in absence of ligand. In this particular situation corepressor complexes actively silence gene expression (Burke & Baniahmad, 2000). A HDAC-containing corepressor complex consisting of the components NcoR (nuclear corepressor)/SMRT (silencing mediator of retinoid and thyroid receptors), mSin3 and RDP3/HDAC1 is displaced by a HAT-containing coactivator complex comprising CBP, p/CAF and SRC-1 (reviewed in (Xu *et al*., 1999)).
Co-crystal structures have revealed that helix 12 of the ligand binding domain of the related estrogen receptor (also harbouring transrepressive properties) adopts a different conformation when bound to agonistic versus antagonistic estrogens (Brzozowski *et al*., 1998; Nichols, Rientjes & Stewart, 1998). Antagonist-bound progesterone receptor and estrogen receptor were further found to interact *in vitro* with the corepressors NcoR and SMRT (Jackson *et al*., 1997; Lavinsky *et al*., 1998; Wagner *et al*., 1998; Zhang *et al*., 1998). Multiple ligands for nuclear receptors are thus capable of influencing the biological activity of the receptor by selectively affecting the recruitment of specific cofactor complexes. The role of each cofactor *in vivo* can be assessed by knockout models of the individual cofactors, such as for SRC-1 (Xu *et al*., 1998) or by cell reconstitution experiments (Lemon *et al*., 2001). Undoubtedly, GR will also recruit its own specific cofactor configuration to enable transactivation. The question is whether there is also a role for corepressor molecules in transrepression mechanisms between NF-κB and GR? GR recruitment of HDAC2 was shown to inhibit interleukin-1β-induced histone acetylation at specific lysine residus (Ito, Barnes & Adcock, 2000). Furthermore, this GR association with HDAC2 *in vivo* could be disrupted by a GR antagonist, mifepristone (Ito *et al*., 2001). A deacetylase inhibitor trichostatine A (TSA) allegedly

demonstrated involvement of histone deacetylase activities in GR-transrepression mechanisms. However, the relative transrepression levels in comparing TNF+DEX+TSA with TNF+TSA where identical to the ones observed without TSA. De facto, these data alone do not allow to conclude on whether deacetylases are involved in the mechanism of transrepression between GR and NF-κB (Vanden Berghe *et al*., 2002). In addition, promoter responsivity to TSA does not necessarily reflect sensitivity to GCs as both IL-8 and HIV promoter activities can be increased by TSA, whereas only IL-8 is responsive to GC repression (Vanden Berghe *et al*., 2002).

5. Future directions

It was mentioned earlier that a GR-mediated Serine phosphorylation switch of RNA polymerase II could lie at the basis of NF-κB and GR cross-talk mechanisms (Nissen & Yamamoto, 2000). It would be of utmost interest to investigate whether this event is promoter-specific or a more general characteristic of GC-mediated repression of NF-κB. It would furthermore be interesting to know whether this mechanism could also account for the reciprocal mechanism. GR is also able to block phosphorylation of CBP, which could be another or additional way by which this transcription factor can modulate gene expression (Perissi *et al*., 1999). Involvement of this modification in cross-repression between NF-κB and GR remains to be explored.

As not only histone tails but also nuclear receptors, NF-κB and cofactors can be acetylated or deacetylated, it will be interesting to learn how these various factor modifications can influence cross-talk mechanisms, in particular between GR and NF-κB.

Similarly, other post-translational modifications may exert their influence. Hormone-dependent histone 3- or histone-4 specific methyltransferases (CARM1 and PRMT1 resp.) have now been characterized in transactivation (Ma *et al*., 2001; Wang *et al*., 2001). Again, not only histones but also CBP/p300 is targeted by CARM1, causing a disturbance of interaction with the transcription factor CREB (Ma *et al*., 2001). A link between these enzymatic activities and GR-NF-κB interplay has not yet been established but will probably be explored in the future.

As pointed out above, chromatin components can be modified by diverse enzymatic activities. Another quite novel concept is the so-called chromatin remodelling, referring to the alteration in the chromatin fiber

structure of a particular nucleosome, or a series of adjacent nucleosomes. To this extent, liganded nuclear receptors may associate with remodelling components SWI/SNF and utilize large ATP-dependent complexes (Kniyamu *et al*., 2000; Urnov & Wolffe, 2001) to bring about these structural changes before any other modifications involved in transcription initiation may occur. Whether the transrepression of p65 by GR and/or the reciprocal transrepression of GR by p65 could work through influencing the chromatin-remodeling machinery needs further experimentation and confirmation *in vivo*.

Finally, we would like to stress the fact that abovementioned models need not be exclusive. A direct protein-protein interaction would not rule out the involvement of cofactors or modulation by chromatin-or factor modifying enzymatic activities such as acetylation, methylation, ubiquitilation, etc. Understanding the subtle regulation and the precise contribution of each of these activities and parameters is of paramount importance to be able to design more specific anti-inflammatory strategies. The aim is to keep the already known great effectiveness of glucocorticoid hormones at place but to get rid of any detrimental side-effects associated with their long-term usage.

Literature

Abbinante-Nissen, J. M., Simpson, L. G. & Leikauf, G. D. (1995). Corticosteroids increase secretory leukocyte protease inhibitor transcript levels in airway epithelial cells. *Am. J. phys.* **268**.

Adcock, I. M. & Caramori, G. (2001). Cross-talk between pro-inflammatory transcription factors and glucocorticoids. *Immunology and Cell Biology* **79,** 376-384.

Adcock, I. M., Nasuhara, Y., Stevens, D. A. & Barnes, P. J. (1999). Ligand-induced differentiation of glucocorticoid receptor (GR) trans-repression and transactivation: preferential targetting of NF-kappaB and lack of I-kappaB involvement. *Br J Pharmacol* **127,** 1003-11.

Adcock, I. M., Newton, R. & Barnes, P. J. (1997). NF-kappa B involvement in IL-1 beta-induction of GM-CSF and COX-2: inhibition by glucocorticoids does not require I-kappa B. *Biochem Soc Trans* **25,** 154s.

Amrani, Y., Lazaar, A. L. & Panettieri, R. A. J. (1999). Up-regulation of ICAM-1 by cytokines in human tracheal smooth muscle cells involves an NF-kappa B-dependent signaling pathway that is only partially sensitive to dexamethasone. *J. Immunol.* **163,** 2128-2134.

Auphan, N., DiDonato, J. A., Rosette, C., Helmberg, A. & Karin, M. (1995). Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis. *Science* **270,** 286-90.

Balsalobre, A., Brown, S. A., Marcacci, L., Tronche, F., Kellendonk, C., Reichardt, H. M., Schütz, G. & Schnibler, U. (2000). Resetting of circadian time in peripheral tissues by glucocorticoid signalling. *Science* **289,** 2344-2347.

Barnes, P. J. (1998). Anti-inflammatory actions of glucocorticoids: molecular mechanisms [editorial]. *Clin Sci Colch* **94,** 557-72.

Barnes, P. J. & Adcock, I. M. (1997). NF-kappa B: a pivotal role in asthma and a new target for therapy. *Trends Pharmacol Sci* **18,** 46-50.

Barnes, P. J., Greening, A. P. & Crompton, G. K. (1995). Glucocorticoid resistance in asthma. *Am J Respir Crit Care Med* **152,** s125-40.

Barnes, P. J. & Karin, M. (1997). Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. *N.Engl.J.Med* **336,** 1066-1071.

Beato, M. (1989). Gene regulation by steroid hormones. *Cell* **56,** 335-344.

Beg, A. A., Sha, W. C., Bronson, R. T., Ghosh, S. & Baltimore, D. (1995). Embryonic lethality and liver degeneration in mice lacking the RelA component of NF-kappa B. *Nature* **376,** 167-170.

Beyaert, R. (1999). NF-kappa B as an emerging target in atopy. *Emerging Therapeutic Targets* **3**.

Beyaert, R., Suffys, P., Van Roy, F. & Fiers, W. (1990). Inhibition by glucocorticoids of tumor necrosis factor-mediated cytotoxicity. Evidence against lipocortin involvement. *FEBS-Letters* **262,** 93-96.

Boumpas, D. T., Chrousos, G. P., Wilder, R. L., Cupps, T. R. & Balow, J. E. (1993). Glucocorticoid therapy for immune-mediated diseases: basic and clinical correlates. *Ann Intern Med* **119,** 1198-208.

Brostjan, C., Anrather, J., Csizmadia, V., Stroka, D., Soares, M., Bach, F. H. & Winkler, H. (1996). Glucocorticoid-mediated repression of NFkappaB activity in endothelial cells does not involve induction of IkappaBalpha synthesis. *J Biol Chem* **271,** 19612-19616.

Brzozowski, A. M., Pike, A. C. W., Dauter, Z., Hubbard, R. E., bonn, T., Engstrom, O., Ohman, L., Greene, G. L., Gustafsson, J. A. & Carlquist, M. (1998). Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature* **389,** 753-758.

Burke, L. J. & Baniahmad, A. (2000). Corepressors. *FASEB J.* **14,** 1876-1888.

Caelles, C., Gonzalez Sancho, J. M. & Muñoz, A. (1997). Nuclear hormone receptor antagonism with AP-1 by inhibition of the JNK pathway. *Genes Dev.* **11,** 3351-3364.

Caldenhoven, E., Liden, J., Wissink, S., Van de Stolpe, A., Raaijmakers, J., Koenderman, L., Okret, S., Gustafsson, J. A. & Van der Saag, P. T. (1995). Negative cross-talk between RelA and the glucocorticoid receptor: a possible mechanism for the antiinflammatory action of glucocorticoids. *Mol Endocrinol* **9,** 401-12.

Cato, A. C. & Wade, E. (1996). Molecular mechanisms of anti-inflammatory action of glucocorticoids. *Bioessays* **18,** 371-8.

Chakravarti, D., LaMorte, V. J., Nelson, M. C., Nakajima, T., Schulman, I. G., Juguilon, H., Montminy, M. & Evans, R. M. (1996). Role of CBP/P300 in nuclear receptor signalling. *Nature* **383,** 99-103.

Chaudhary, L. R. & Avioli, L. V. (1996). Regulation of interleukin-8 gene expression by interleukin-1beta, osteotropic hormones, and protein kinase inhibitors in normal human bone marrow stromal cells. *J Biol Chem* **271,** 16591-6.

De Bosscher, K., Schmitz, M. L., Vanden Berghe, W., Plaisance, S., Fiers, W. & Haegeman, G. (1997). Glucocorticoid-mediated repression of nuclear factor-kappaB-dependent transcription involves direct interference with transactivation. *Proc Natl Acad Sci U S A* **94,** 13504-13509.

De Bosscher, K., Vanden Berghe, W. & Haegeman, G. (2000a). Mechanisms of anti-inflammatory action and of immunosuppression by glucocorticoids: negative interference of activated glucocorticoid receptor with transcription factors. *J. Neuroimmunology* **109,** 16-22.

De Bosscher, K., Vanden Berghe, W. & Haegeman, G. (2001). Glucocorticoid repression of AP-1 is not mediated by competition for nuclear coactivators. *Molecular Endocrinology* **15,** 219-227.

De Bosscher, K., Vanden Berghe, W., Vermeulen, L., Plaisance, S., Boone, E. & Haegeman., G. (2000b). Glucocorticoids repress NF-kB-driven genes by disturbing the interaction of p65 with the basal transcription machinery, irrespective of coactivator levels in the cell. *Proc. Natl. Acad. Sci.* **97,** 3919-3924.

De Kloet, E. R., Vreugdenhil, E., Oitzl, M. S. & Joels, M. (1998). Brain corticosteroid receptor balance in health and disease. *Endocr Rev* **19,** 269-301.

De Vera, M. E., Taylor, B. S., Wang, Q., Shapiro, R. A., Billiar, T. R. & Geller, D. A. (1997). Dexamethasone suppresses iNOS gene expression by upregulating I-kappa B alpha and inhibiting NF-kappa B. *Am J Physiol* **273,** g1290-6.

DeFranco, D. B. & Guerrero, J. (2000). Nuclear matrix targeting of steroid receptors: specific signal sequences and adaptor proteins. *Crit. Rev. Eukaryot. Gene Expr.* **10,** 39-44.

Delany, A. M., Gabbitas, B. Y. & Canalis, E. (1995). Cortisol downregulates osteoblast alpha 1 (I) procollagen mRNA by transcriptional and posttranscriptional mechanisms. *J Cell Biochem* **57,** 488-94.

Doucas, V., Shi, Y., Miyamoto, S., West, A., Verma, I. & Evans, R. M. (2000). Cytoplasmic catalytic subunit of protein kinase A mediates cross-repression by NF-kappa B and the glucocorticoid receptor. *Proc. Natl. Acad. Sci* **97,** 11893-11898.

Doucas, V., Tini, M., Egan, D. A. & Evans, R. M. (1999). Modulation of CREB binding protein function by the promyelocytiv (PML) oncoprotein suggests a role for nuclear bodies in hormone signalling. *Proc. Natl. Acad. Sci* **96,** 2627-2632.

Eggert, M., Mows, C. C., Tripier, D., Arnold, R., Michel, J., Nickel, J., Schmidt, S., Beato, M. & Renkawitz, R. (1995). A fraction enriched in a novel glucocorticoid receptor-interacting protein stimulates receptor-dependent transcription in vitro. *J Biol Chem* **270,** 30755-9.

Francastel, C., Schubeler, D., Martin, D. I. & Groudine, M. (2000). Nuclear compartmentalization and gene activity. *Nat. Rev. Mol. Cell Biol.* **1,** 137-143.

Gerritsen, M. E., Williams, A. J., Neish, A. S., Moore, S., Shi, Y. & Collins, T. (1997). CREB-binding protein/p300 are transcriptional coactivators of p65. *Proc Natl Acad Sci U.S.A.* **94,** 2927-2932.

Hager, G. L., Lim, C. S., Elbi, C. & Baumann, C. T. (2000). TRafficking of nuclear receptors in living cells. *J. Steroid Biochem. Mol. Biol.* **74,** 249-254.

Heck, S., Bender, K., Kullmann, M., Gottlicher, M., Herrlich, P. & Cato, A. C. (1997). I kappaB alpha-independent downregulation of NF-kappaB activity by glucocorticoid receptor. *EMBO J* **16,** 4698-707.

Heck, S., Kullmann, M., Gast, A., Ponta, H., Rahmsdorf, H. J., Herrlich, P. & Cato, A. C. (1994). A distinct modulating domain in glucocorticoid receptor monomers in the repression of activity of the transcription factor AP-1. *EMBO J* **13,** 4087-4095.

Helmberg, A., Auphan, N., Caelles, C. & Karin, M. (1995). Glucocorticoid-induced apoptosis of human leukemic cells is caused by the repressive function of the glucocorticoid receptor. *Embo J* **14,** 452-60.

Hinz, M., Krappmann, D., Eichten, A., Heder, A., Scheidereit, C. & Strauss, M. (1999). NF-kappa B function in growth control: regulation of cyclin D expression and G0/G1-to-S-phase transition. *Mol Cell Biol* **19,** 2690-2698.

Hofmann, T. G., Hehner, S. P., Bacher, S., Droge, W. & Schmitz, M. L. (1998). Various glucocorticoids differ in their ability to induce gene expression, apoptosis and to repress NF-kappaB-dependent transcription. *FEBS Lett* **441,** 441-6.

Horwitz, K. B., Jackson, T. A., Bain, D. I., Richer, J. K., Takimoto, G. K. & Tung, L. (1996). Nuclear receptor coactivators and corepressors. *Mol Endocrinol* **10,** 1167-1177.

Ito, K., Barnes, P. J. & Adcock, I. M. (2000). Glucocorticoid receptor recruitment of histone deacetylase 2 inhibits IL-1beta-induced histone H4 acetylation on Lysines 8 and 12. *Mol. Cell. Biol.* **20,** 6891-6903.

Ito, K., Jazrawi, E., Cosio, B., Barnes, P. J. & Adcock, I. M. (2001). p65-activated histone acetyltransferase activity is repressed by glucocorticoids: mifepristone fails to recruit HDAC2 to the p65-HAT complex. *J. Biol. Chem.* **276,** 30208-30215.

Jackson, T. A., Richer, J. K., Bain, D. L., Takimoto, G. S., Tung, L. & Horwitz, K. B. (1997). The partial agonist activity of antagonist-occupied steroid receptors is controlled by a novel hinge domain-binding coactivator L7/SPA and the corepressors N-CoR or SMRT. *Mol Endocrinol* **11,** 693-705.

Jehn, B. M. & Osborne, B. A. (1997). Gene regulation associated with apoptosis. *Crit. Rev. Eukaryot.Gene Expr.* **7,** 179-193.

Kaltschmidt, B., Kaltschmidt, C., Hehner, S. P., Dröge, W. & Schmitz, M. L. (1999). Repression of NF-kappa B impairs HeLa cell proliferation by functional interference with cell cycle checkpoint regulators. *Oncogene* **18**.

Kamei, Y., Xu, L., Heinzel, T., Torchia, J., Kurokawa, R., Gloss, B., Lin, S. C., Heyman, R. A., Rose, D. W., Glass, C. K. & Rosenfeld, M. G. (1996). A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. *Cell* **85,** 403-414.

Karin, M. (1998). New twists in gene regulation by glucocorticoid receptor: is DNA binding dispensable? *Cell* **93,** 487-490.

Kassel, O., Sancono, A., Krätzschmar, J., Kreft, B., Stassen, M. & Cato, C. B. (2001). Glucocorticoids inhibit MAP kinase via increased expression and decreased degradation of MKP-1. *EMBO J* **20,** 7108-7116.

Kniyamu, H. K., Fryer, C. J., Horwitz, K. B. & Archer, T. K. (2000). The mouse mammary tumor virus promoter adopts distinct chromatin structures in hulan breast cancer cells with and without glucocorticoid receptor. *J. Biol. Chem.* **275,** 20061-20068.

Korzus, E., Torchia, J., Rose, D. W., Xu, L., Kurokawa, R., McInerney, E. M., Mullen, T. M., Glass, C. K. & Rosenfeld, M. G. (1998). Transcription factor-specific requirements for coactivators and their acetyltransferase functions. *Science* **279,** 703-707.

Lasa, M., Brook, M., Saklatvala, J. & Clark, R. A. (2001). Dexamethasone destabilizes cyclooxygenase 2 mRNA by inhibiting mitogen-activated protein kinase p38. *Mol. Cell. Biol.* **21,** 771-780.

Lavinsky, R. M., Jepsen, K., Heinzel, T., Torchia, J., Mullen, T. M., Schiff, R., Del Rio, A. L., Ricote, M., Ngo, S., Gemsch, J., Hilsenbeck, S. G., Osborne, C. K., Glass, C. K., Rosenfeld, M. G. & Rose, D. W. (1998). Diverse signaling pathways modulate nuclear receptor recruitment of N-CoR and SMRT complexes. *Proc Natl Acad Sci U S A* **95,** 2920-2925.

Lefstin, J. A. & Yamamoto, K. R. (1998). Allosteric effects of DNA on transcriptional regulators. *Nature* **392,** 885-888.

Lemon, B., Inouye, C., King, D. S. & Tjian, R. (2001). selectivity of chromatin-remodelling cofactors for ligand-activated transcription. *Nature* **414,** 924-928.

Lemon, B. & Tjian, T. (2000). Orchestrated response: a symphony of transcription factors for gene control. *Genes Dev* **14,** 2551-2569.

Lezoualc'h, F., Sagara, Y., Holsboer, F. & Behl, C. (1998). High constitutive NF-kappaB activity mediates resistance to oxidative stress in neuronal cells. *J Neurosci* **18,** 3224-32.

Liden, J., Delaunay, F., Rafter, I., Gustafsson, J. & Okret, S. (1997). A new function for the C-terminal zinc finger of the glucocorticoid receptor. Repression of RelA transactivation. *J Biol Chem* **272,** 21467-72.

Liden, J., Rafter, I., Truss, M., Gustafsson, J. & Okret, S. (2000). Glucocorticoid effects on NF-kappa B binding in the transcription of the ICAM-1 gene. *Biochem Biophys Res Commun* **273,** 1008-1014.

Ma, H., Baumann, C. T., Li, H., Strahl, B. D., Rice, R., Jelinek, M. A., Aswad, D. W., Allis, C. D. & Stallcup, M. R. (2001). hormone-dependent, CARM1-directed, arginine-specific methylation of histone H3 on a steroid-regulated promoter. *Curr Biol.* **11,** 1981-1985.

McEwan, I. J., Wright, A. P. & Gustafsson, J. A. (1997). Mechanism of gene expression by the glucocorticoid receptor: role of protein-protein interactions. *Bioessays* **19,** 153-160.

McKay, L. I. & Cidlowski, J. A. (1998). Cross-talk between nuclear factor-kappa B and the steroid hormone receptors: mechanisms of mutual antagonism. *Mol Endocrinol* **12,** 45-56.

Meyer, T., Carlstedt Duke, J. & Starr, D. B. (1997). A weak TATA box is a prerequisite for glucocorticoid-dependent repression of the osteocalcin gene. *J Biol Chem* **272,** 30709-14.

Moras, D. & Gronemeyer, H. (1998). The nuclear receptor ligand-binding domain: structure and function. *CURRENT.OPINION.IN CELL BIOLOGY.* **10,** 384-391.

Newton, R., Hart, L. A., Stevens, D. A., Bergmann, M., Donnelly, L. E., Adcock, I. M. & Barnes, P. J. (1998). Effect of dexamethasone on interleukin-1beta-(IL-1beta)-induced nuclear factor-kappaB (NF-kappaB) and kappaB-dependent transcription in epithelial cells. *Eur J Biochem* **254,** 81-9.

Nichols, M., Rientjes, J. M. & Stewart, A. F. (1998). Different positioning of the ligand-binding domain helix 12 and the F domain of the estrogen receptor accounts for functional differences between agonists and antagonists. *EMBO J* **17,** 765-73.

Nissen, R. M. & Yamamoto, K. R. (2000). The glucocorticoid receptor inhibits NFkappaB by interfering with serine-2 phosphorylation of the RNA polymerase II carboxy-terminal domain. *Genes Dev* **14,** 2314-2329.

Onate, S. A., Tsai, S. Y., Tsai, M. J. & O'Malley, B. W. (1995). Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. *Science* **270,** 1354-1357.

Oro, A. E., Hollenberg, S. M. & Evans, R. M. (1988). Transcriptional inhibition by a glucocorticoid receptor-beta-galactosidase fusion protein. *Cell* **55,** 1109-1114.

Perissi, V., Dasen, J. S., Kurokawa, R., Wang, Z. Y., Korzus, E., Rose, D. W., Glass, C. K. & Rosenfeld, M. G. (1999). Factor-specific modulation of CREB-binding protein acetyltransferase activity. *PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA* **96,** 3652-3657.

Perkins, N. D. (1997). Achieving transcriptional specificity with NF-kB. *Int J Biochem Cell Biol* **29,** 1433-1448.

Ramdas, J. & Harmon, J. M. (1998). Glucocorticoid-induced apoptosis and regulation of NF-kappaB activity in human leukemic T cells. *Endocrinology* **139,** 3813-21.

Ray, K. P. & Searle, N. (1997). Glucocorticoid inhibition of cytokine-induced E-selectin promoter activation. *Biochem Soc Trans* **25,** 189s.

Ray, P., Ghosh, S. K., Zhang, D. H. & Ray, A. (1997). Repression of interleukin-6 gene expression by 17 beta-estradiol: inhibition of the DNA-binding activity of the transcription factors NF-IL6 and NF-kappa B by the estrogen receptor. *FEBS Lett* **409,** 79-85.

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

Reichardt, H. M., Tuckermann, J. P., Göttlicher, M., Vujic, M., Weih, F., Angel, P., Herrlich, P. & Schütz, G. (2001). Repression of inflammatory responses in the absence of DNA binding by the glucocorticoid receptor. *EMBO J* **20,** 7168-7173.

Resche-Rigon, M. & Gronemeyer, H. (1998). Therapeutic potential of selective modulators of nuclear receptor action. *Curr. Op. Chem. Biol.* **2,** 501-507.

Scheinman, R., Cogswell, P. C., Lofquist, A. & Baldwin, A. S. (1995a). Role of transcriptional activation of i kappa b alpha in mediation of immunosuppression by glucocorticoids [see comments]. *Science* **270,** 283-286.

Scheinman, R. I., Gualberto, A., Jewell, C. M., Cidlowski, J. A. & Baldwin, A. S., Jr. (1995b). Characterization of mechanisms involved in transrepression of NF- kappa B by activated glucocorticoid receptors. *Mol Cell Biol* **15,** 943-953.

Schmitz, M. L., Stelzer, G., Altmann, H., Meisterernst, M. & Baeuerle, P. A. (1995). Interaction of the COOH-terminal transactivation domain of p65 NF-kappa B with TATA-binding protein, transcription factor IIB, and coactivators. *J Biol Chem* **270,** 7219-7226.

Sheppard, K.-A., Phelps, K. M., Williams, A. J., Thanos, D., Glass, C. K., Rosenfeld, M. G., Gerritsen, M. E. & Collins, T. (1998). Nuclear integration of glucocorticoid receptor and nuclear factor-kappaB signaling by CREB-binding protein and steroid receptor coactivator-1. *J Biol Chem* **273,** 29291-4.

Sheppard, K.-A., Rose, D. W., Haque, Z. K., Kurokawa, R., McInerney, E., Westin, S., Thanos, D., Rosenfeld, M. G., Glass, C. K. & Collins, T. (1999). Transcriptional activation by NF-kappa B requires multiple coactivators. *Mol Cell Biol* **19,** 6367-6378.

Stenoien, D. L., Mancini, M. G., Patel, K., Allegretto, E. A., Smith, C. L. & Mancini, M. A. (2000). Subnuclear trafficking of estrogen receptor alpha and steroid receptor coactivator-1. *Mol. Endocrinol.* **15,** 518-534.

Stewart, S. & Crabtree, G. R. (2000). Transcription. Regulation of the regulators. *Nature* **408,** 46-47.

Swantek, J. L., Cobb, M. H. & Geppert, T. D. (1997). Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) is required for lipopolysaccharide stimulation of tumor necrosis factor alfa (TNF-a) translation: glucocorticoids inhibit TNF-afla translation by blocking JNK/SAPK. *Mol Cell Biol* **17,** 6274-6282.

Tao, Y., Williams-Skipp, C. & Scheinman, R. I. (2001). Mapping of glucocorticoid receptor DNA binding domain surfaces contributing to transrepression of NF-kappaB and induction of apoptosis. *J. Biol. Chem.* **276**.

Thiele, K., Bierhaus, A., Autschbach, F., Hofmann, M., Stremmel, W., Thiele, H., Ziegler, R. & Nawroth, P. P. (1999). Cell specific effects of glucocorticoid treatment on the NF-kappa Bp65/IkappaBalpha system in patients with Crohn's disease. *Gut* **45,** 693-704.

Tobler, A., Meier, R., Seitz, M., Dewald, B., Baggiolini, M. & Fey, M. F. (1992). Glucocorticoids downregulate gene expression of GM-CSF, NAP-1/IL-8, and IL-6, but not of M-CSF in human fibroblasts. *Blood* **79,** 45-51.

Unlap, M. T. & Jope, R. S. (1997). Dexamethasone attenuates NF-kappa B DNA binding activity without inducing I kappa B levels in rat brain in vivo. *Brain Res Mol Brain Res* **45,** 83-9.

Urnov, F. D. & Wolffe, A. P. (2001). A necessary good: nuclear hormone receptors and their chromatin template. *Mol. Endocrinol.* **15,** 1-16.

Vanden Berghe, W., De Bosscher, K., Plaisance, S., Boone, E. & Haegeman, G. (1999a). The Nuclear Factor-kappa B engages CBP/p300 and Histone Acetyltransferase Activity for Transcriptional Activation of the Interleukin-6 Gene Promoter. *J Biol Chem* **274,** 32091-32098.

Vanden Berghe, W., De Bosscher, K., Vermeulen, L., De Wilde, G. & Haegeman, G. (2002). Induction and repression of NF-kappa B driven inflammatory genes. *Springer-Verlag Series* **In Press**.

Vanden Berghe, W., Francesconi, E., De Bosscher, K., Rèsche-Rigon, M. & Haegeman, G. (1998). Dissociated Glucocorticoids with Antiinflammatory Potential Repress Interleukin-6 Gene Expression by an NF-kappa-B-Dependent Mechanism. *submitted* .

Vanden Berghe, W., Francesconi, E., De Bosscher, K., Rèsche-Rigon, M. & Haegeman, G. (1999b). Dissociated Glucocorticoids with Antiinflammatory Potential Repress Interleukin-6 Gene Expression by an NF-kappa-B-Dependent Mechanism. *Mol Pharmacol* **56,** 797-806.

Wagner, B. L., Norris, J. D., Knotts, T. A., Weigel, N. L. & McDonnell, D. P. (1998). The nuclear corepressors NCoR and SMRT are key regulators of both ligand- and 8-bromo-cyclic AMP-dependent transcriptional activity of the human progesterone receptor. *Mol Cell Biol* **18,** 1369-78.

Wang, H., Huang, Z. Q., Xia, L., Feng, Q., Erdjument-Bromage, H., Strahl, B. D., Briggs, S. D., Allis, C. D., Wong, J., Tempst, P. & Zhang, Y. (2001). Methylation of Histone H4 at arginine 3 facilitating transcriptional activation by nuclear hormone receptors. *Science* **293,** 853-857.

Wissink, S., van Heerde, E. C., Schmitz, M. L., Kalkhoven, E., van der Burg, B., Baeuerle, P. A. & van der Saag, P. T. (1997). Distinct domains of the RelA NF-kappaB subunit are required for negative cross-talk and direct interaction with the glucocorticoid receptor. *J Biol Chem* **272,** 22278-84.

Wissink, S., van Heerde, E. C., vand der Burg, B. & van der Saag, P. T. (1998). A dual mechanism mediates repression of NF-kappaB activity by glucocorticoids. *Mol Endocrinol* **12,** 355-63.

Wolffe, A. & Pruss, D. (1996). Targeting chromatin disruption: transcription regulators that acetylate histones. *Cell* **84,** 817-819.

Wolffe, A. P. (1997). Transcriptional control. Sinful repression. *Nature* **387,** 16-7.

Wolffe, A. P., wong, J. & Pruss, D. (1997). Activators and repressors: making use of chromatin to regulate transciption. *Genes to Cells* **2,** 291-302.

Xu, J., Qiu, Y., DeMayo, F. J., Tsai, S., Tsai, M. & O'Malley, B. W. (1998). Partial hormone resistance in mice with disruption of the steroid receptor coactivator-1 (SRC-1) gene. *Science* **279,** 1922-1925.

Xu, L., Glass, C. K. & Rosenfeld, M. G. (1999). Coactivator and corepressor complexes in nuclear receptor function. *Curr Op Gen Dev* **9,** 140-147.

Young, J. D., Lawrence, A. J., MacLean, A. G., Leung, B. P., McInnes, I. B., Canas, B., Pappin, D. J. C. & Stevenson, R. D. (1999). Thymosin beta sulfoxide is an anti-inflammatory agent generated by monocytes in the presence of glucocorticoids. *Nature Med* **5,** 1424-1427.

Zhang, X., Jeyakumar, M., Petukhov, S. & Bagchi, M. K. (1998). A nuclear receptor corepressor modulates transcriptional activity of antagonist-occupied steroid hormone receptor. *Mol Endocrinol* **12,** 513-24.

Zhong, H., Voll, R. E. & Ghosh, S. (1998). Phosphorylation of NF-kappa B p65 by PKA stimulates transcriptional activity by promoting a novel bivalent interaction with the coactivator CBP/p300. *Mol Cell* **1,** 661-671.