

From DEPARTMENT OF MEDICAL NUTRITION  
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**GLUCOCORTICOID RECEPTOR  
CROSS-TALK WITH NF- $\kappa$ B AND AP-1:**  
Functional role and mechanisms

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STOCKHOLM 2005

Published and printed by Larserics Digital Print AB  
Box 20082, SE-161 02 Bromma, Sweden  
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ISBN 91-7140-324-8

*To my family*

## ABSTRACT

The protein investigated in this thesis is the glucocorticoid receptor (GR), which is a ligand activated transcription factor that belongs to the superfamily of nuclear hormone receptors. The GR contains three functional independent domains, the N-terminal domain containing a major transactivation region, the central DNA binding domain (DBD) and the C-terminal domain that binds the hormone/ligand. Glucocorticoid hormones, which bind to and activate the intracellular located GR, are steroid hormones that are involved in several important physiological processes such as metabolism, inflammatory and immune responses and cell proliferation. The GR exerts its effects by activating and repressing transcriptional activity of target genes. Repression of target genes in many cases involves interference with the activity of the transcription factors NF- $\kappa$ B and AP-1, a process usually referred to as cross-talk.

Results presented in studies I-II show that GR cross-talk with NF- $\kappa$ B and AP-1 signaling pathways can be separated. Using a model system devoid of endogenous functional GR (HEK293 cells), it was possible to study effects of different GR mutants. It was demonstrated that a point mutation in the C-terminal zinc-finger of the DBD impaired the receptor's ability to repress NF- $\kappa$ B but not AP-1. Using this 'loss-of-function' mutant it was revealed that inhibition of activated extracellular signal-regulated kinase (ERK), is an important determinant for functional GR mediated repression of NF- $\kappa$ B. In wild type GR, but not GR mutant containing cells, induction of the endogenous ERK inhibitor MKP-1 was observed, highlighting this glucocorticoid mediated mechanism to be important for NF- $\kappa$ B repression in contexts where ERK is activated. In conclusion, GR cross-talk with NF- $\kappa$ B and AP-1 can be separated. This allowed us using gene expression profiling to identify endogenous genes preferentially repressed by GR interference with NF- $\kappa$ B.

Glucocorticoids exert an antiproliferative effect on most cells. However, the molecular mechanisms are still largely unclear. In study III, the antiproliferative mechanism by glucocorticoids is further investigated using GR mutants that discriminate between cross-talk with NF- $\kappa$ B and AP-1, transactivation and transrepression and antiproliferative versus non- antiproliferative responses. Gene expression profiling revealed that the NF- $\kappa$ B inhibitor, I $\kappa$ B $\alpha$ , was upregulated in cells displaying an antiproliferative response to glucocorticoids. Unexpectedly, the induction of I $\kappa$ B $\alpha$  was shown to be a glucocorticoid response element (GRE) independent mechanism. Selective expression of I $\kappa$ B $\alpha$  demonstrated that I $\kappa$ B $\alpha$  up-regulation was sufficient for the antiproliferative effect. In summary, this demonstrates that glucocorticoid inhibition of NF- $\kappa$ B is an important mechanism in the antiproliferative response to glucocorticoids.

In HeLa cells the antiproliferative mechanism by glucocorticoids has been demonstrated to involve a direct transcriptional induction of the cyclin dependent kinase inhibitor p57<sup>Kip2</sup>. In study IV the promoter region of p57<sup>Kip2</sup> was further investigated. These studies identified a functional GRE in the human p57<sup>Kip2</sup> promoter, located 5 kb upstream of the transcriptional start site. The GRE was shown to be well conserved in the mouse, both regarding sequence similarity and function, indicating a high biological relevance for this element.

Keywords: glucocorticoid receptor, glucocorticoids, cross-talk, NF- $\kappa$ B, AP-1, cell cycle, cell proliferation

## LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to by their Roman numerals:

- I** **Bladh, L. G.**, J. Lidén, K. Dahlman-Wright, M. Reimers, S. Nilsson, and S. Okret 2005. Identification of endogenous glucocorticoid repressed genes differentially regulated by a glucocorticoid receptor mutant able to separate between NF- $\kappa$ B and AP-1 repression  
*Mol Pharmacol*, 67 (3):815-826. Epub 2004 Nov 18.
- II** **Bladh, L. G.**, I. Rafter, S. Nilsson, and S. Okret 2005. Glucocorticoid mediated repression of NF- $\kappa$ B activity requires inhibition of extracellular signal-regulated kinase (ERK)  
*Manuscript*.
- III** **Bladh, L. G.**, J. Lidén, A. Pazirandeh, I. Rafter, K. Dahlman-Wright, S. Nilsson, and S. Okret 2005. Identification of Target Genes Involved in the Antiproliferative Effect of Glucocorticoids Reveals a Role for Nuclear Factor- $\kappa$ B Repression  
*Mol Endocrinol*. **19**:632-43 Epub 2004 Nov 04.
- IV** Alheim, K., J. Corness, M. K. Samuelsson, **L. G. Bladh**, T. Murata, T. Nilsson, and S. Okret 2003. Identification of a functional glucocorticoid response element in the promoter of the cyclin-dependent kinase inhibitor p57<sup>Kip2</sup>  
*J Mol Endocrinol*. **30**:359-68.

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## LIST OF ABBREVIATIONS

ACTH	Adrenocorticotrophic hormone
AP-1	Activator protein-1
AR	Androgen receptor
CBG	Corticosteroid binding globulin
CBP	CREB binding protein
CDK	cyclin-dependent kinase
ChIP	Chromatin immunoprecipitation
CKI	CDK inhibitor
COUP-TF	Chicken ovalbumin upstream promoter-transcription factor
CREB	cAMP-response element-binding protein
CRH	Corticotropin-releasing hormone
DBD	DNA binding domain
DRIP	Vitamin D receptor interacting protein
ELL	Eleven-nineteen lysine rich leukemia
ER	Estrogen receptor
ERR	Estrogen-related receptor
ERK	Extracellular signal-regulated kinase
FKBP	FK506 binding protein
FRAP	Fluorescence recovery after photobleaching
GR	Glucocorticoid receptor
GRE	Glucocorticoid response element
GRU	Glucocorticoid response unit
HAT	Histone acetyltransferase
HDAC	Histone deacetylase
HPA-axis	Hypothalamus-pituitary-adrenal axis
Hsp	Heat shock protein
ICAM-1	Intercellular adhesion molecule-1
IL	Interleukin
JNK	c-Jun N-terminal kinase
LBD	Ligand binding domain
MAPK	Mitogen-activated protein kinase
MKP-1	MAPK phosphatase-1
MR	Mineralocorticoid receptor
MSK1	mitogen- and stress-activated protein kinase-1
NCoR	Nuclear receptor corepressor
NF- $\kappa$ B	Nuclear factor-kappaB
PEPCK	Phosphoenolpyruvate carboxykinase
PIC	Pre-initiation complex

PKA	Protein kinase A
POMC	Pro-opiomelanocortin
PPAR	Peroxisome proliferator-activated receptor
PXR	Pregnane X receptor
RAR	Retinoic acid receptor
RXR	Retinoid X receptor
TAF	TBP associated factor
TBP	TATA box binding protein
TNF $\alpha$	Tumor necrosis factor alpha
TR	Thyroid hormone receptor
TRAP	Thyroid hormone receptor associated protein
VDR	Vitamin D receptor
11 $\beta$ -HSD	11 beta-hydroxysteroid dehydrogenase



## INTRODUCTION

### REGULATION OF TRANSCRIPTION

During physiological conditions a cell is exposed to a multitude of signals and depending on its specific function, the cell integrates these signals into an ordered response by either up- or downregulating expression of appropriate genes. This process, by which a gene's encoded information is decoded into a protein, is referred to as gene expression. First the DNA strand information is transcribed into RNA, which is transferred to the cytoplasm where the information is translated into a protein by ribosomes. The DNA region located immediately upstream of the transcription start site is termed core promoter. The core promoter consists of an AT-rich region, termed TATA box, located approximately 30 base-pairs upstream of the transcription start site. In eukaryotic cells transcription of protein-encoding genes is mediated by RNA polymerase II, which require general transcription factors that recognize core promoter sequences to properly target RNA polymerase II to the promoter. The initial step is binding of the general transcription factor TFIID, consisting of the TATA box binding protein (TBP) and TBP associated factors (TAFs). This is followed by recruitment of RNA polymerase II together with other general transcription factors that forms a large multiprotein complex required to initiate transcription termed pre-initiation complex (PIC). Subsequently, phosphorylation of the C-terminal domain (CTD) of RNA polymerase facilitates promoter clearance and progression into elongation (Woychik and Hampsey, 2002).

Polymerase II mediated transcription is further regulated by binding of specific transcription factors, such as nuclear receptors, to distinct regulatory elements located in the promoter or enhancer regions. Enhancer regions can be located at great distances, either up- or downstream, of the promoter. These specific transcription factors in turn, facilitate recruitment of additional proteins and protein complexes to the promoter that change the compact chromatin structure (see below) and make

contacts with the general transcription machinery. The series of events initiated by specific transcription factors allows formation of PIC and transcriptional activation.

The chromatin structure constitutes an additional level of regulation of gene transcription. While a compact and organized chromatin structure inhibits binding of regulatory proteins that activate gene expression, remodeled chromatin is less compact and more accessible to regulatory proteins and thus allows activation of gene expression. The basic structural element of chromatin, the nucleosome, is organized in arrays together with DNA. A nucleosome consists of approximately 146 bp of DNA wrapped around an octamer of histones containing two molecules each of four core histones, H2A, H2B, H3 and H4 (Luger et al., 1997). Alteration of this ordered structure leaves the DNA more accessible to proteins such as transcription factors and the general transcription machinery. Notably, nuclear receptors represents a group of specific transcription factors that have the capacity to bind to their recognition elements in the DNA despite the fact that the chromatin matrix is normally in a compact and relatively inaccessible conformation (Hebbar and Archer, 2003).

In order to induce transcription, specific transcription factors such as nuclear receptors utilize coregulators to decompact the organized chromatin structure. Coregulators can be defined as cellular factors recruited by specific transcription factors that complement their function as regulators of gene expression. Coregulators that promote gene expression are termed coactivators and those that inhibit promoter activity are termed corepressors. Coactivators in turn, can broadly be divided into two categories, ATP dependent chromatin remodeling proteins and histone modifying proteins. Both categories have the capacity to modify the chromatin structure and thereby facilitate gene expression. The ATP dependent chromatin remodeling complexes disrupt the nucleosomal structure and increase DNA accessibility in a non-covalent manner (Narlikar et al., 2002). In contrast, histone modifying enzymes alter chromatin structure through posttranslational covalent modifications, such as acetylation, phosphorylation and methylation (Fischle et al., 2003). Functions associated with corepressors, on the other hand, in many ways contrasts those of coactivators. In the case of nuclear receptor dependent gene expression, corepressor proteins that contain histone deacetylase (HDAC) activity are recruited to DNA by the receptors and

thereby negatively regulate gene expression (Kinyamu and Archer, 2004; Perissi et al., 1999).

Another transcriptional coregulator termed Mediator, consists of a large multisubunit complex that make contacts both with gene-specific activators and the general transcriptional machinery at the promoter to support gene expression (Malik and Roeder, 2000). The Mediator complex also referred to as thyroid hormone receptor associated protein (TRAP)/vitamin D receptor interacting protein (DRIP) complex, is important for ligand dependent transcriptional activation by nuclear receptors (Rachez et al., 1999).

Many of the factors involved in gene expression, such as the general transcription machinery, specific transcription factors and coregulators are common factors that in many cases are ubiquitously expressed. Still, regulation of gene expression displays a high degree of specificity. What then determines the specificity? Obviously, cell type specific expression of signaling molecules including transcription factors affects gene expression for a given cell or tissue. The endpoint of many signal transduction pathways is the activation of specific transcription factors. Given that the regulatory regions of genes contain binding sites for multiple transcription factors, allow genes to respond to multiple signaling pathways. Accordingly, different stimulators impose gene expression specificity by activating specific subsets of factors involved in gene regulation. Transport of regulatory proteins between cytoplasmic and nuclear compartments represents another way of regulating the access of transcriptional regulators to DNA. Posttranslational modification such as phosphorylation may initiate a change in protein compartment localization. Further, phosphorylation may also modulate the intrinsic activity of both transcription factors and coregulators. The transcription factors have in many cases different abilities to interact with coregulators. This is of importance with regard to transcription factors that recognize the same regulatory element, e.g. steroid receptors (see below). Although binding to the same regulatory element, the recruitment of different coregulators leads to different levels of gene expression (Smith and O'Malley, 2004). An additional level of signaling complexity is possible in that two transcription factors that recognize the same regulatory element may be differently modulated by the same coregulator. For

example, the elongation factor Eleven-nineteen lysine rich leukemia (ELL) was found to function as a coregulator. While ELL increased gene expression by the mineralocorticoid receptor (MR), ELL decreased glucocorticoid receptor (GR) mediated gene expression (Pascual et al., 2005). Moreover, the Mediator that is comprised of multiple proteins opens up the possibility of variation in complex composition that is formed in response to signals from transcription factors (Woychik and Hampsey, 2002). Collectively, this combinatorial and context dependent regulation allows cells to respond to a diverse array of stimuli using the same factors. Consequently, the level of transcription at a specific gene is determined by the composition of active regulatory factors recruited.

## **NUCLEAR RECEPTORS**

Nuclear receptors are transcription factors that are involved in virtually all biological processes, such as reproduction, development and metabolism (Beato et al., 1995; Mangelsdorf et al., 1995). These receptors function primarily as ligand activated transcription factors and thereby provide a direct link between the signaling molecule (ligand) and transcriptional regulation. The ligands, which are small lipophilic molecules, passively diffuse through the cell membrane and bind to the intracellular located receptor. In addition to ligand activation, which induces a conformational change of the receptor, the activity of nuclear receptors can be further modified by at least two additional mechanisms. One mechanism involves covalent modifications of the receptor, such as phosphorylations and the other involves interaction with other proteins e.g. other transcription factors and coregulators.

Based on sequence homology analysis of the two most conserved parts of the receptors, the DNA- and ligand binding domains, nuclear receptors constitute a superfamily of transcription factors that in humans consist of 48 members (Gronemeyer et al., 2004). Although endogenous ligands for several of these receptors have been identified, there are receptors that have no known ligand and these are referred to as 'orphan' nuclear receptors. This large group of proteins can be classified into separate subfamilies based on the source and type of their cognate ligand (classical

receptors, adopted orphan receptors and orphan receptors (Chawla et al., 2001)), dimerization and DNA binding properties (steroid receptors, retinoid X receptor (RXR) heterodimers and orphan receptors (Mangelsdorf et al., 1995)) or evolution (six subfamilies (Laudet, 1997)). Based on the latter classification, a unified nomenclature system for the nuclear receptor superfamily has been endorsed by many researchers within the field (Committee, 1999). Using this nomenclature, the official name for the nuclear receptor investigated in this thesis, the glucocorticoid receptor (GR) is NR3C1.

According to the evolutionary analysis of the receptors that led to six different subfamilies, a large subfamily denoted NR1 is formed by thyroid hormone receptor (TR), retinoic acid receptor (RAR), vitamin D receptor (VDR) and peroxisome proliferator-activated receptor (PPAR) as well as different orphan receptors. A second subfamily, NR2, includes the RXRs and a number of orphan receptors. The RXRs play an important role in nuclear receptor signaling as they function as partners for receptors that bind DNA as heterodimers. Receptors forming subfamily NR3 includes the steroid nuclear receptors, i.e. the glucocorticoid receptor (GR), mineralocorticoid receptor (MR), progesterone receptor (PR), androgen receptor (AR) and the estrogen receptor (ER), as well as the orphan receptor estrogen-related receptor (ERR). The steroid receptor NR3 subfamily, in contrast to receptors in the NR1 class, has not been reported to heterodimerize with RXR, rather they bind 'head-to-head' to DNA as homodimers. Though, there are reports suggesting that GR-MR heterodimers can be formed (Liu et al., 1995; Trapp et al., 1994). The remaining subfamilies of the human nuclear receptor family consist of orphan receptors, which bind DNA either as homodimers, heterodimers or monomers.

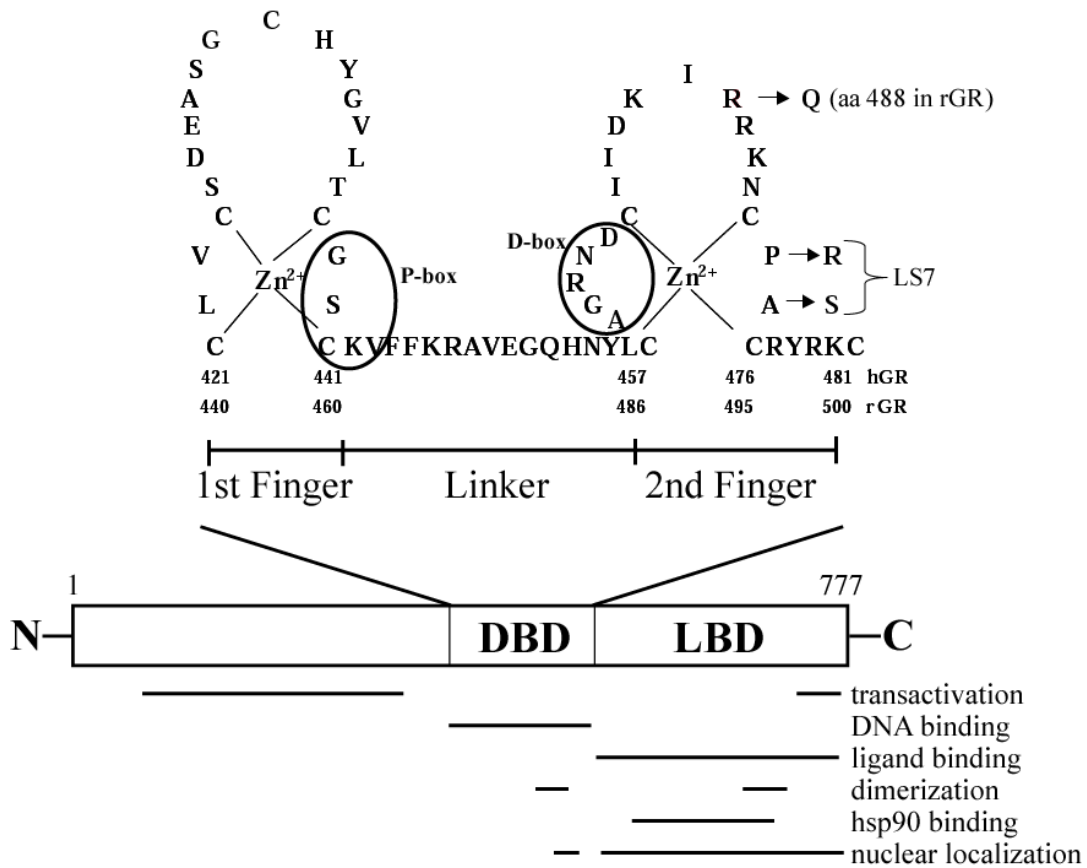
The fact that nuclear receptors play an important role in a broad range of biological processes and several of these receptors are regulated by small lipophilic substances have led to a large interest in these proteins as pharmaceutical targets. For example, ER is an important factor involved in breast cancer growth and therefore may be treated by ER antagonists (e.g. tamoxifen), PPAR $\gamma$  is targeted with agonists such as troglitazone for the treatment of type 2 diabetes and for the treatment of inflammatory disorders the GR is targeted by GR agonists, e.g. dexamethasone. In addition, the relatively large group of receptors with an as yet unknown ligand, the so-called orphan receptors, is a

potential opportunity for drug discovery. However, it should be noted that it is not known whether all orphan receptors have the potential to bind ligand. For example, the orphan receptor Nurr1, most probably does not bind any ligand. Crystallographic studies have revealed that the ligand binding pocket is occupied with hydrophobic amino acid side chains that prohibit interaction with any ligand (Wang et al., 2003). Noteworthy, the opposite is also true, there are nuclear receptors that have a large and promiscuous binding pocket. For example, the PPARs and pregnane X receptor (PXR) which primarily function as metabolic and xenobiotic sensors, respectively, have large binding pockets and bind structurally diverse ligands (Benoit et al., 2004).

### **Domain structure and function**

Proteins within the superfamily of nuclear receptors share a common modular structure comprised of three independent but interacting functional domains. In the following discussion these domains are exemplified by the GR domain structure and function (Fig.1). Proteolysis experiments using purified GR revealed the modular structure of GR and demonstrated three functionally independent domains, including an N-terminal immunoreactive domain, a central DNA binding domain and a C-terminal ligand binding domain (Carlstedt-Duke et al., 1982; Wrange and Gustafsson, 1978). Later on the cloning of human, rat and mouse GR was performed, which confirmed the earlier data and greatly facilitated further exploration of the GR's structure and function (Danielsen et al., 1986; Hollenberg et al., 1985; Miesfeld et al., 1984; Miesfeld et al., 1986). In addition to the general structure of transcription factors, which consists of a transactivation domain and a DNA binding domain, nuclear receptors such as GR, also contain a ligand binding domain. A splice variant of the GR termed GR $\beta$  has also been identified, which is truncated at the C-terminus, and as a consequence, cannot bind glucocorticoid hormone (Hollenberg et al., 1985). Therefore, GR $\alpha$  is sometimes used to denote the longer glucocorticoid-binding version of GR. The physiological role of the truncated GR $\beta$  isoform is unclear. While there are reports suggesting that GR $\beta$  negatively influences the activity of GR $\alpha$ , other studies cannot detect any activity of the GR $\beta$  (Carlstedt-Duke, 1999; Vottero and Chrousos, 1999). The expression levels of the GR $\beta$  variant most often are considerably lower than GR $\alpha$ , indicating a limited influence on GR $\alpha$  signaling. To date, the relevance of GR $\beta$  *in vivo* is still controversial

and will not be further discussed in this thesis. GR $\alpha$  is denoted GR throughout the remainder of this thesis.



**Figure 1.** Schematic view of the GR domain structure and function (lower part of the picture). Upper part illustrates a magnification of the DBD region with its linker and two zinc-fingers. The P- and D-box are highlighted (circles) together with different GR mutants (arrows).

### *N-terminal domain*

The amino terminus, also known as the A/B-domain, contains a transactivation region (AF-1) that interacts with factors/complexes involved in chromatin remodeling e.g. Brahma-related gene-1 (BRG1), cAMP-response element-binding protein (CREB) binding protein (CBP) and p300/CBP-associated factor (P/CAF) (Jenkins et al., 2001). This region has also been shown to interact with the TBP (subunit of TFIID) of the general transcription machinery (Ford et al., 1997). Additionally, posttranslational modification such as phosphorylation of serine residues have been demonstrated to take place in this domain by two major kinase families, the mitogen-activated protein

kinases (MAPKs) and cyclin-dependent kinases (CDKs), and thereby contribute to modulate GR activity (Ismaili and Garabedian, 2004). Although increased phosphorylation has been demonstrated in this region following hormone treatment, functional analysis using GR mutants unable to become phosphorylated only revealed a modest effect on the receptor's transcriptional activity (Almlöf et al., 1995). The influence of GR phosphorylation on transcriptional activation, however, has later been suggested to be promoter dependent (Webster et al., 1997). Interestingly, different phosphorylated isoforms of GR localize to distinct subcellular compartments and the transcriptional activity of GR correlates with increased phosphorylation at a distinct residue, serine 211 (Wang et al., 2002). Thus, apart from being hyperphosphorylated following ligand activation, the functional consequences following phosphorylation with regard to GR transcriptional regulation remains largely elusive.

#### *Ligand binding domain*

The carboxy terminus of nuclear receptors contains the ligand binding domain (LBD or E-domain). Similar to amino terminus, this region contains a transactivation function (AF-2) that, in contrast to AF-1, is ligand dependent. A family of coactivator proteins, often referred to as p160s (SRC-1, SRC-2/TIF2/GRIP1 and SRC-3/ACTR/RAC3/pCIP/AIB1), interact with AF-2 that is formed within the LBD of GR in an agonist dependent, antagonist sensitive manner (Anzick et al., 1997; Onate et al., 1995; Voegel et al., 1996) ((Smith and O'Malley, 2004) and references therein). These coactivators interact with nuclear receptors through a domain that contains multiple LXXLL motifs, in which L represents leucine and X denotes any amino acid (Heery et al., 1997). During the second half of 1990s the LBD structure for several nuclear receptors was elucidated revealing an overall structural similarity ((Aranda and Pascual, 2001) and references therein). Accordingly, the AF-2 and its core region consisting of helices 3, 5 and 12 represent a conserved part of the LBD. Helix 12 is relatively flexible and adopts different positions that are agonist/antagonist dependent. Not until recently, the GR LBD was crystallized and structurally determined, both in complex with agonist and antagonist (Bledsoe et al., 2002; Kauppi et al., 2003). Binding of the agonist dexamethasone induces a conformational change where helix 12 adopts a position over the ligand binding pocket, forming a surface that allows coactivator interaction. Thus, the ligand functions as an allosteric regulator shifting GR from one state to another. In



contrast to agonist binding, GR binding of the antagonist RU486 physically prevents helix 12 from adopting its agonist conformation. Instead, helix 12 adopts a conformation that partially occupies the region in space where the coactivator resides when complexed with the nuclear receptor, and thus prevents coactivator binding (Kauppi et al., 2003). This antagonist-induced conformation of the receptor allows interaction with corepressors such as nuclear receptor corepressor (N-CoR) (Schulz et al., 2002; Stevens et al., 2003). Structural studies performed with PPAR $\alpha$  indicate that nuclear receptors distinguish between coregulators by the different lengths of interaction motifs within the two types of coregulators, coactivator ('short') and corepressor ('long') (Xu et al., 2002). Dictated by the type of ligand, agonist or antagonist, respectively, different sized groves are formed. Accordingly, antagonists prevent helix 12 to adopt its active position resulting in a larger pocket that can accommodate the longer motif of the corepressor. There are also nuclear receptors that interact with corepressors in the absence of ligand, e.g. TR and RAR, and thereby repress basal transcription (Chen and Evans, 1995; Perissi et al., 1999). Binding of the ligand results in replacement of the corepressors by coactivators, this leads to transcriptional activation.

#### *DNA binding domain*

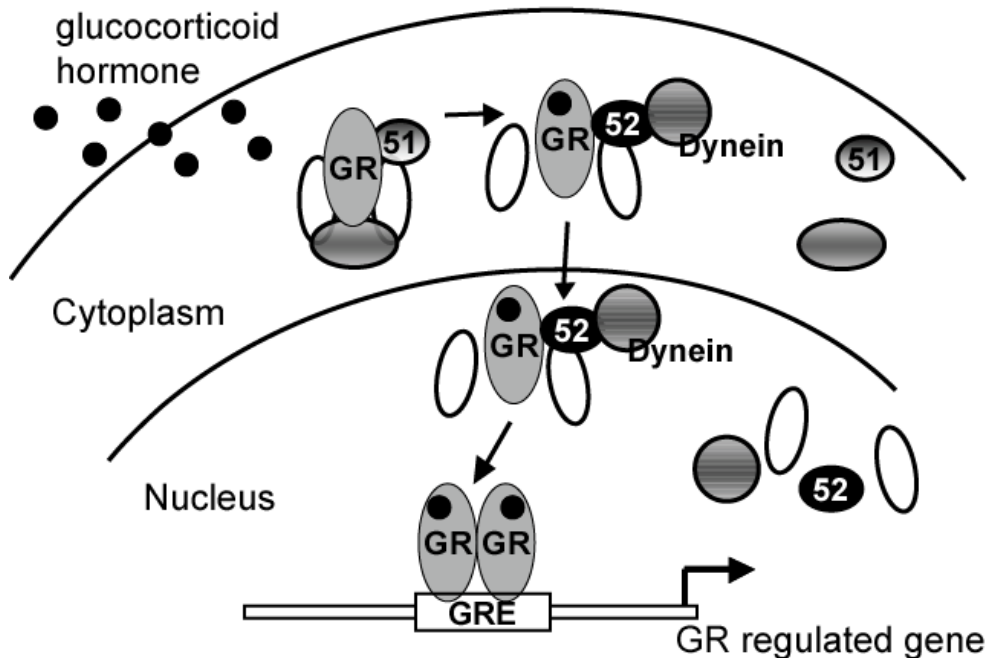
The central DNA binding domain (DBD or the C-domain), is responsible for recognizing specific sequences within the genome known as glucocorticoid responsive elements (GREs). A GRE consists of two hexameric half-sites spaced by three nucleotides that form a palindromic sequence. The consensus sequence derived from comparison of functional GREs in target genes reads 5'-GGTACAnnnTGTTCT-3' (Zilliacus et al., 1995). A common feature among the steroid receptors is that they bind to palindromic REs as homodimers, where each DBD motif contacts one of the hexameric sites in the RE. Actually, the same sequence is recognized by the GR, MR, PR and AR, whereas ER binds to a distinct, but related palindromic sequence. Two zinc-fingers motifs constitute the DBD, where each finger binds a zinc ion that is coordinated by four cystein residues. The N-terminal zinc-finger is mainly involved in site-specific recognition, where amino acids within the P-box are of great importance (Zilliacus et al., 1995). A different sub-region in the DBD is denoted the D-box and is located in the C-terminal zinc-finger. This region functions as a dimer interface and, as

a consequence, mutations in this region result in a receptor unable to bind DNA as a dimer (Dahlman-Wright et al., 1991; Luisi et al., 1991). In addition to its crucial function as a DNA interaction surface, the GR DBD region has also been demonstrated to be important for the receptor's interference with NF- $\kappa$ B and AP-1, possibly via protein-protein interactions (see below). Functions assigned to different domains are shown in Figure 1. While the amino terminus is variable both with regard to length and amino acid sequence, the DBD and LBD are highly conserved among the nuclear receptors and for that reason the last two domains are commonly used for sequence homology analysis.

### **Mechanism of GR action**

As briefly mentioned, nuclear receptors function as ligand activated transcription factors. In the case of unliganded GR, the receptor is held in a high affinity state for its ligand by chaperone proteins, such as heat shock protein (hsp) 90 and hsp70 and cochaperones such as p23 and immunophilins (Pratt and Toft, 1997). In this state, the receptor is mainly localized in the cytoplasm. Upon glucocorticoid binding the receptor becomes hyperphosphorylated, immunophilins are exchanged where FK506 binding protein (FKBP)-51 is substituted by FKBP52 and concomitant recruitment of the transport protein dynein, but leaving the hsp90 unchanged (Davies et al., 2002; Pratt et al., 2004). In contrast to what was thought for many years, the receptor is now believed to translocate into the nucleus in complex with hsp90, FKBP52 and dynein. Thus, instead of inducing a cytoplasmic chaperone-free receptor, binding of ligand is thought to convert the receptor from a state of relatively persistent complex with hsp90 to a state that is more dynamic. Following nuclear translocation, the GR binds as a homodimer to GREs in promoter regions of target genes and regulates gene transcription (Fig.2). As described in more detail in a previous section, this regulation of transcription involves recruitment of chromatin remodeling complexes and coactivators and thereby facilitates binding of both specific and general transcription factors. The DNA-bound GR communicates directly or indirectly, via coactivators, with the general transcriptional machinery to regulate polymerase II-directed transcription.

In line with the interaction between GR and molecular chaperones being dynamic and that it takes place both in the cytoplasm and nucleus, molecular chaperones may participate in various stages of GR trafficking. For example, treatment of cells with an hsp90 inhibitor, geldanamycin, results in a decreased nuclear GR movement, in addition to a decreased translocation of GR from cytoplasm to nucleus (Elbi et al., 2004; Galigniana et al., 1998). Moreover, there is support for involvement of molecular chaperones in receptor nuclear recycling, which include p23 and hsp90 mediated disassembly of GR containing transcriptional regulatory complexes and removal of GR from the DNA (Freeman and Yamamoto, 2002; Liu and DeFranco, 1999). Given the rapid (minutes) and selective effects observed following GR activation, an efficient delivery of the receptor to the gene-specific sequences should occur. Together, molecular chaperones may participate in various stages of GR trafficking, such as nuclear translocation, movements within the nucleus and receptor exchange from specific response elements.



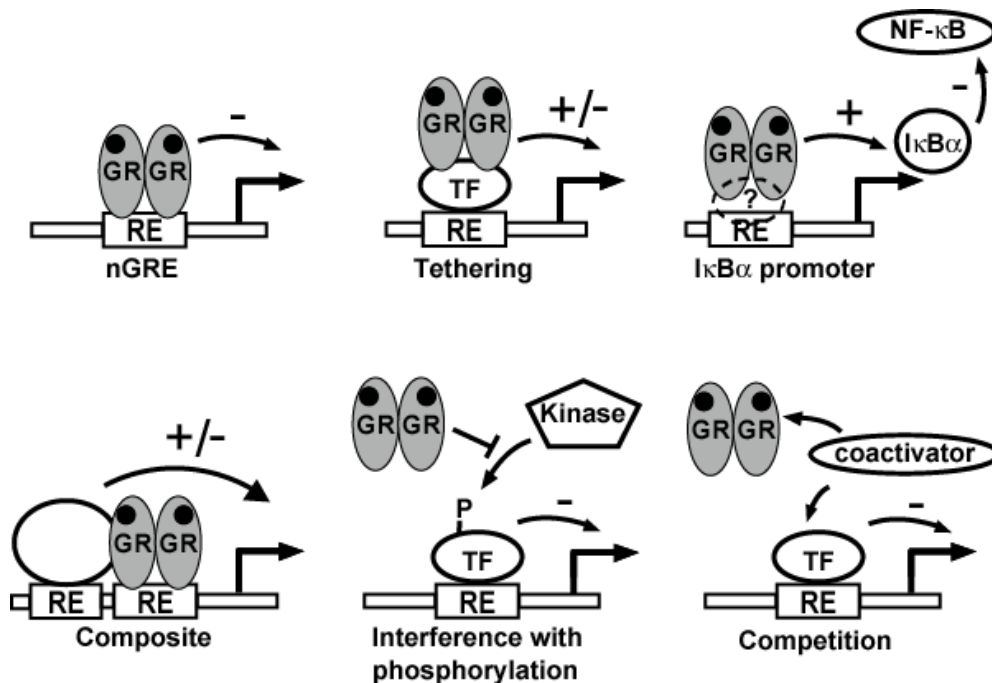
**Figure 2.** Schematic illustration of the classical activation process of GR.

The growing appreciation for the involvement of highly dynamic interactions in the regulation of nuclear receptor mediated transcription comes from studies making use of recent technological advances. For example, the use of a biochemical approach, chromatin immunoprecipitation, revealed a cyclic association of both the ER and coregulators with endogenous promoters (Shang et al., 2000). It was observed that promoter occupancy by ER and p160 coactivators had similar periodicities and was paralleled with histone acetylation. More recently, a new approach, fluorescence recovery after photobleaching (FRAP), has made it possible to measure protein mobility in live cells (Walker et al., 1999). Using this system it is possible to study the dynamics of binding of the receptor to the DNA in live cells. Based on this technique, the GR has been recorded to rapidly come on and off the multiple GRE containing DNA in a time dependent manner and therefore a 'hit and run' model has been proposed (McNally et al., 2000). Although the different techniques display a dynamic interaction between nuclear receptors and DNA, there is still a discrepancy with regard to the time frame in which these interactions occur. With reference to the chromatin immunoprecipitation methodology, the cycles are in the order of minutes, whereas the FRAP approach detects cycles in the order of seconds. Anyway, this model of dynamic GR interplay with both DNA and regulatory complexes enables the receptor to monitor and respond efficiently to changes in hormone levels.

There are, in addition to the preceding illustration of GR involvement in the up-regulation of gene expression, GR binding sites in glucocorticoid responsive genes that negatively influence gene expression. These responsive elements are referred to as negative GREs (nGREs), which similar to GREs require direct binding of the GR (Fig.3). Consequently, an nGRE recognition sequence resembles that of a GRE, although the consensus sequence of an nGRE is more variable than the sequence forming a GRE (Truss and Beato, 1993). Examples of genes reported to harbor nGREs in their promoter regions are the glucocorticoid-regulated genes coding for osteocalcin, pro-opiomelanocortin (POMC) and prolactin (Drouin et al., 1989; Strömstedt et al., 1991; Subramaniam et al., 1997).

### Glucocorticoid receptor cross-talk mechanisms

In addition to the classical mode of action by nuclear receptors described above involving GR-DNA interaction, a second mode of action is based on the receptor's interplay with other signaling pathways, an interplay that is often referred to as cross-talk. Since the term cross-talk is not well defined, further discussion relating to cross-talk will represent positive or negative interaction between different signaling pathways that have an effect on gene transcription. Over the years, several mechanisms have been suggested to explain how the GR cross-talks with other transcription activators. Many of the studies investigating glucocorticoid actions include GR cross-talk with two well-studied transcription activators, nuclear factor (NF)- $\kappa$ B and activator protein (AP)-1. The fact that no reports so far have demonstrated nGREs to be involved in the negative cross-talk between the GR and the two transcription factors, NF- $\kappa$ B and AP-1, other mechanisms most probably are involved. Summarized below are a number of cross-talk mechanisms that have been described in the literature (Fig.3).



**Figure 3.** Illustration of molecular mechanisms of non-classical GR actions.

*Tethering model*

Most studies tend to favor a so-called tethering mechanism where GR indirectly targets DNA via protein-protein interaction with other transcription factors bound to their cognate DNA-binding sites. For the most part, this interaction results in a negative influence on gene transcription, e.g. GR negative cross-talk with the transcription factors AP-1 and NF- $\kappa$ B (De Bosscher et al., 2003). In vitro experiments have shown a direct protein-protein interaction between GR and the two transcription factors, in line with the tethering mechanism (Caldenhoven et al., 1995; Jonat et al., 1990; Nissen and Yamamoto, 2000; Wissink et al., 1997; Yang-Yen et al., 1990). Interestingly, although direct DNA binding of the GR is not involved in tethering, the DBD of the GR still seems to participate in this mechanism (Heck et al., 1994; Jonat et al., 1990; Lidén et al., 1997; Schüle et al., 1990; Yang-Yen et al., 1990). Thus, in addition to GRE mediated transcription where obviously the DBD is involved, this highlights the relevance of the DBD in relation to GR cross-talk with other signaling pathways. Accordingly, we have utilized GR DBD mutants to investigate the GR cross-talk with NF- $\kappa$ B and AP-1 in more detail.

The well recognized mutual cross-talk between the GR and AP-1 was one of the first described (Jonat et al., 1990; Schüle et al., 1990; Yang-Yen et al., 1990). In line with the tethering model, coimmunoprecipitation experiments suggested a direct protein-protein association between the GR and AP-1 (Jonat et al., 1990). Subsequently further support was obtained by genomic footprinting studies demonstrating that the AP-1 binding to DNA was unaffected although an efficient glucocorticoid repression of AP-1 activity was displayed (König et al., 1992). Recently, the negative cross-talk between GR and AP-1 was further scrutinized by Rogatsky and colleagues (Rogatsky et al., 2001). By performing chromatin immunoprecipitations they could demonstrate that the GR was tethered to the AP-1 complex still bound to the collagenase-3 promoter. Interestingly, they also showed that the before mentioned coactivator TIF2/GRIP1 was recruited to the promoter by GR, but instead of activating transcription TIF2/GRIP1 potentiated GR mediated repression in the presence of agonist but not antagonist. This recruitment seems to be specific to GR, since parallel experiments with the TR $\beta$  did not lead to TIF2/GRIP1 occupancy at the promoter, although TR $\beta$  repressed AP-1

activity comparable to GR. A possible mechanism for the receptor to reduce the transcriptional activity downstream of AP-1 binding could be via decreasing histone acetylations at the promoter. However, no decline in histone acetylations was detected nor was the GR mediated AP-1 repression sensitive to the HDAC inhibitor trichostatin A, thus suggesting that recruitment of HDAC is not likely to be involved in GR mediated repression of AP-1 at the collagenase-3 promoter. Further studies are required in order to identify the mechanism explaining these results.

NF- $\kappa$ B dependent gene expression represents another signaling pathway that is suggested to be repressed by GR in a GRE independent manner. Transrepression of NF- $\kappa$ B by glucocorticoids in many ways resembles that of AP-1. Again, direct protein-protein interaction between the GR and NF- $\kappa$ B has been demonstrated and the repression is mutual, i.e. NF- $\kappa$ B is able to inhibit GR activity (Caldenhoven et al., 1995; Nissen and Yamamoto, 2000; Ray and Prefontaine, 1994; Wissink et al., 1997). Similar to AP-1, NF- $\kappa$ B dependent activation can be inhibited by GR while NF- $\kappa$ B remains bound to its response element in the promoter region. Studies utilizing the two *in vivo* approaches, genomic footprinting and chromatin immunoprecipitation, suggest that GR associates to the NF- $\kappa$ B complex at the promoter, rather than displacing NF- $\kappa$ B from the DNA (Ito et al., 2000; Lidén et al., 2000; Nissen and Yamamoto, 2000). Interestingly, two different mechanisms were suggested, yet both involved posttranslational modifications. One mechanism involved GR interference with serine-2 phosphorylation of the RNA polymerase II C-terminal domain. Although glucocorticoid treatment repressed NF- $\kappa$ B dependent signaling, it did not impede NF- $\kappa$ B complex recruitment to known NF- $\kappa$ B regulated promoters, interleukin-8 (IL-8) and intercellular adhesion molecule-1 (ICAM-1). In addition, GR repression of NF- $\kappa$ B activity was resistant to trichostatin A, suggesting HDAC recruitment to be a less likely mechanism. Remarkably, GR did not inhibit PIC assembly, but rather interfered with phosphorylation of serine-2 of the RNA polymerase II (Nissen and Yamamoto, 2000). Possible explanations for the unphosphorylated serine-2 of polymerase II could be that GR, via steric hindrance, inhibits recruitment of a kinase or GR could recruit a factor that dephosphorylates serine-2. Clearly further studies are required in order to explain the mechanism in more detail. The other mechanism was reported by a group

studying the NF- $\kappa$ B regulated granulocyte-macrophage colony-stimulating factor promoter (Ito et al., 2000). In contrast to the mechanism described above, a GR dependent reduction of acetylation of histones correlating to inhibition of CBP associated HAT activity and the recruitment of HDAC2 was reported. Perhaps different promoters require different repressive mechanisms by the GR and no single mechanism applies to all cells or genes regulated by NF- $\kappa$ B and repressed by GR.

Regulation of gene promoters by GR interaction with other transcription factors without a direct GR DNA interaction sometimes lead to increased gene transcription. For example, the GR enhance transcription of the  $\beta$ -casein promoter via a functional interaction between the receptor and the transcription factor signal transducer and activator of transcription-5 (STAT5) (Stöcklin et al., 1996). In addition, glucocorticoid mediated activation of Hoxb-1 gene expression has been suggested to involve protein-protein interaction between the GR and Pbx1 protein (Subramaniam et al., 2003). Finally, the GR potentiates transcription from the herpes simplex virus thymidine kinase promoter via a GRE independent mechanism. It is suggested that the mediated effect through a functional interaction between the GR and the CCAAT enhancer-binding protein  $\beta$  (Boruk et al., 1998). The reason why GR interaction with some transcription factors results in repression and in some cases upregulation is not clear.

#### *I $\kappa$ B $\alpha$ upregulation*

In relation to glucocorticoid repression of NF- $\kappa$ B, it has also been suggested that glucocorticoids induce expression of the NF- $\kappa$ B inhibitor, I $\kappa$ B $\alpha$  (Auphan et al., 1995; Scheinman et al., 1995). These results were obtained in cells such as cervical carcinoma HeLa cells, monocytes and T-lymphocytes. In contrast, other studies have reported results arguing against this mechanism. An I $\kappa$ B $\alpha$  independent glucocorticoid mediated repression of NF- $\kappa$ B has been shown in a diverse set of other cell types, e.g. osteoblast U2OS cells, kidney epithelial cell line NRK-52E cells and aortic endothelial BAEC cells (Brostjan et al., 1996; Ohtsuka et al., 1996; Stein and Yang, 1995). This indicates that glucocorticoid mediated I $\kappa$ B $\alpha$  upregulation is important in some cell types but not in others. Further, it has been reported that GR mutants, in addition to being unable to activate transcription via a GRE dependent mechanism, can dissociate



NF- $\kappa$ B repression from I $\kappa$ B $\alpha$  induction, thus indicating the two glucocorticoid mediated effects to be separate phenomena (Heck et al., 1997). Notably, to date no GREs have been identified in the I $\kappa$ B $\alpha$  promoter, implicating a GRE independent mechanism to be involved in cells where glucocorticoids induce I $\kappa$ B $\alpha$  synthesis. Possibly the receptor targets the DNA indirectly via binding to an already DNA bound factor, so-called tethering (see above). In line with this, we have identified a GR mutant unable to activate transcription through a GRE dependent mechanism, while still being able to induce I $\kappa$ B $\alpha$  expression (Bladh et al., 2005). Additionally, another nuclear receptor, PPAR $\alpha$ , which also lacks a functional response element in the I $\kappa$ B $\alpha$  promoter, is capable of activating I $\kappa$ B $\alpha$  expression (Delerive et al., 2002; Delerive et al., 2000). Here, it was demonstrated that intact binding sites for NF- $\kappa$ B and SP-1 within the I $\kappa$ B $\alpha$  promoter were required, thus opening up the possibility for PPAR $\alpha$  to utilize one or both of these transcription factors to indirectly target and activate the promoter. Similarly, based on the importance of a NF- $\kappa$ B binding site in the I $\kappa$ B $\alpha$  promoter, the lack of GRE and the well-recognized interaction between GR and NF- $\kappa$ B, a putative mechanism for GR mediated I $\kappa$ B $\alpha$  expression could be via protein-protein interaction between the receptor and NF- $\kappa$ B. This, however, remains to be confirmed, in particular since it would be in contrast to the frequently observed inhibitory effect by glucocorticoids on NF- $\kappa$ B dependent signaling.

#### *Composite elements*

GR composite elements contain binding sites for both the GR and for factors that are essential for receptor activity. Thus, GR interacts with these DNA elements, in contrast to the models of cross-talk described above that most probably do not involve direct binding of GR to DNA. The activity of the receptor is determined by the other factors that bind to the composite element. One example is a glucocorticoid-induced gene encoding a key enzyme involved in gluconeogenesis, the phosphoenolpyruvate carboxykinase (PEPCK) gene. The promoter region of the PEPCK gene contains a composite element, which is often referred to as glucocorticoid response unit (GRU) (Imai et al., 1990). This GRU is comprised of two non-consensus GR binding sites and at least three accessory factor elements that bind hepatic nuclear factor 4 (HNF4), HNF3 $\beta$  and chicken ovalbumin upstream promoter-transcription factor (COUP-TF),

which are believed to facilitate GR binding to the PEPCK promoter (Stafford et al., 2001). Interestingly, the coactivator PGC-1, which is induced by CREB and glucocorticoids, is believed to induce PEPCK expression through the GRU, and thus possibly constitutes a positive feedback loop during conditions of high activity/levels of CREB and glucocorticoids, such as fasting and stress (Herzig et al., 2001; Yoon et al., 2001). The proliferin gene promoter is another example of a regulatory region containing such an element. In this case it is a composite GRE-AP-1 site, referred to as plfG. Depending on the composition of the AP-1 complex that activates the gene, either a positive (AP-1, Jun/Jun) or negative (AP-1, Fos/Jun) glucocorticoid response is conferred (Diamond et al., 1990). Moreover, although both GR and MR can bind to the plfG, only GR is able to repress AP-1 stimulated promoter activity, whereas the MR is inactive. Interestingly, an N-terminal region of the GR seems to be required for the repressive effect at plfG, since introduction of this region into the MR reconstituted the ability of MR to repress via the plfG (Pearce and Yamamoto, 1993).

#### *GR interference with transcription factor phosphorylation*

Previous sections describing GR cross-talk models have all concerned events that for the most part take place in the nucleus. This section will briefly discuss cross-talk mechanisms that also can take place in the cytoplasm. Stimulation of cellular signaling cascades will ultimately lead to activation of transcription factors, an activation that often involves phosphorylation of the proteins. For example, a report demonstrating protein kinase A catalytic subunit (PKAc) to physically interact with and phosphorylate the p65 subunit of NF- $\kappa$ B, supports an important role of PKAc in p65 mediated transactivation (Zhong et al., 1997). More recently, Doucas and colleagues demonstrated that GR interferes with PKAc-p65 interaction and suggested this to be a mechanism by which GR transrepresses NF- $\kappa$ B dependent signaling (Doucas et al., 2000). Support for an additional mechanism involving GR interference with phosphorylation of a transcription factor has been reported where GR represses AP-1 activity by interfering with phosphorylation of AP-1 (Caelles et al., 1997). In this case, the c-Jun N-terminal kinase (JNK) MAPK pathway is involved. JNK potentiates the activity of AP-1 by phosphorylating one of the major components of AP-1, c-Jun, at serine 63 and 73. Hormone activated GR is suggested to downmodulate JNK enzyme activity via physical interaction with the enzyme (Bruna et al., 2003).

### *Competition model*

The activation of genes by transcription factors involves recruitment of cofactors (see above). Similar to GR, the cofactors CBP and SRC-1 have been shown to enhance transcriptional activation of both NF- $\kappa$ B and AP-1 (Arias et al., 1994; Gerritsen et al., 1997; Lee et al., 1998; Na et al., 1998). Based on these observations, a hypothetical model explaining the repressive actions by glucocorticoids has been proposed. This model involves competition between GR and the driving transcription factors, NF- $\kappa$ B or AP-1, for a limited amount of CBP or SRC-1, in the cell (Kamei et al., 1996; Sheppard et al., 1998). Later on, however, other reports have demonstrated that glucocorticoid mediated repression of NF- $\kappa$ B and AP-1 is independent of CBP and SRC-1 levels (De Bosscher et al., 2001; De Bosscher et al., 2000; Wu et al., 2004a). Instead, CBP may act via facilitating the interaction between GR and NF- $\kappa$ B rather than acting as a cofactor for which both transcription factors compete (McKay and Cidlowski, 2000).

## **GLUCOCORTICIDS**

Glucocorticoids are small lipophilic steroid hormones that are produced in the adrenal cortex. More specifically, glucocorticoids are synthesized from cholesterol in the zona fasciculata and zona reticularis of the adrenal cortex by a series of cytochrome P450-catalyzed reactions (Findling et al., 1997). The synthesis and secretion of glucocorticoids, in human cortisol and in rodent corticosterone, are mainly regulated by the hypothalamus-pituitary-adrenal axis (HPA-axis). Corticotropin-releasing hormone (CRH), produced in hypothalamus in a diurnal rhythm that causes a peak early in the morning, stimulates the release of adrenocorticotrophic hormone (ACTH). ACTH in turn, produced and released in the anterior pituitary, rapidly induces production and secretion of glucocorticoids from the adrenal cortex. In addition to CRH stimulation under the control of diurnal rhythm, many stresses stimulate CRH release, often superseding the normal diurnal rhythmicity. As a result of being controlled by CRH, glucocorticoid levels are high in the morning and during stress.

Importantly, the HPA-axis is negatively regulated by glucocorticoids via a feedback loop involving glucocorticoid action on both the pituitary and hypothalamus levels.

### **Biological effects of glucocorticoids**

Based on the fact that the GR is ubiquitously expressed, it is reasonable to assume that glucocorticoids have an effect on nearly all cells in the body. The name glucocorticoid derives from one of the first functions of glucocorticoids that were identified, namely its effects on glucose metabolism. Regulation of circulating glucose levels by glucocorticoids is mainly through enhanced gluconeogenesis in the liver and inhibited glucose up-take by peripheral tissues, e.g. muscle and adipose tissue. Further, they also slow down nucleic acid and protein synthesis and increase protein catabolism. These metabolic effects are believed to form the basis for the unwanted effects that are accompanied with long-term glucocorticoid treatment and may lead to e.g. precipitation of diabetes. Importantly, glucocorticoids also inhibit inflammatory and immune responses. Although upregulation of genes with repressive effect on inflammation exist, the antiinflammatory and immunospressive effect by glucocorticoids is for the most part believed to occur via inhibition of proinflammatory genes (De Bosscher et al., 2003). A diverse set of genes coding for proteins involved at different levels of the inflammatory response have been identified, including cytokines, chemokines, enzymes and adhesion molecules, which ensure an efficient inhibition of inflammatory and immune responses.

Further evidence highlighting the biological importance of glucocorticoids comes from animal models. The *in vivo* relevance of glucocorticoid signaling during development can be seen in knockout mice, in which the GR gene has been deleted. These mice die shortly after birth due to respiratory failure (Cole et al., 1995). Interestingly, this phenotype can be reversed by expression of a dimerization deficient GR mutant termed GRdim, in which a point mutation in the D-box has been introduced, that leads to impaired transactivation via GREs (Reichardt et al., 1998). Accordingly, this suggests that glucocorticoid mediated transactivation through classical GREs is dispensable for normal development and homeostasis in mice, which further highlights the significance of glucocorticoid mediated cross-talk with other signaling pathways. Indeed, similar to control mice that express the wild type receptor, GRdim mice

display a functional glucocorticoid induced repression of proinflammatory genes following inflammatory stimuli (Reichardt et al., 1998; Reichardt et al., 2001). Furthermore, the GRdim mice have provided additional information relating to the negative feedback mechanism of the HPA-axis. While CRH expression and ACTH release is unaltered, expression of the larger ACTH precursor molecule pro-opiomelanocortin (POMC) is altered in GRdim mice, thus further demonstrating the complexity of different modes of action by GR (Reichardt and Schütz, 1998). Interestingly, some genes may still be activated in the GRdim mice. For instance, the GRdim mutant has been demonstrated to upregulate the GR dependent phenylethanolamine *N*-methyltransferase (PNMT) gene in vitro (Adams et al., 2003). Moreover, since the GRdim mice display an unaltered PNMT expression, while GR knockout mice lack PNMT expression, the in vivo animal models provide further support for the in vitro observation (Reichardt et al., 1998).

### **Glucocorticoid sensitivity**

Glucocorticoid sensitivity can be modulated at nearly every level of the glucocorticoid-signaling pathway. Factors affecting glucocorticoid sensitivity includes availability of the hormone, tissue-specific factors, intracellular metabolism of the hormone and variations in the receptor protein. Obviously the availability of hormone dictates the degree of GR activity. The majority of circulating glucocorticoid hormones in the blood are bound to corticosteroid-binding globulin (CBG) and to a lesser extend to albumin. Given that it is the free fraction of hormone that can bind and activate the GR, CBG binding regulates the sensitivity by directing the access to and provides supply for the hormone. During stressful conditions such as septic disorder, the CBG level drops due to an interleukin-6 (IL-6) dependent hepatic posttranslational blockade. As a consequence, the free fraction of hormone increases, which in turn suppresses the inflammatory response (Pugeat et al., 1989; Tsigos et al., 1998). Moreover, the intracellular availability of glucocorticoids can also be modulated. Cortisol does not only bind to and activate GR, it also activates MR. In tissues expressing both GR and MR, e.g. kidney, the cortisol is converted to its inactive form cortisone by 11 $\beta$ -hydroxysteroid dehydrogenase type 2 (11 $\beta$ -HSD2) making this tissue less sensitive to glucocorticoids, but enables aldosterone, that only binds to MR, to exert its mineralocorticoid effect (Andrews and Walker, 1999). By contrast, the 11 $\beta$ -HSD1

isoform converts inactive cortisone to active cortisol and has been reported to be expressed in tissues such as liver, fat and in the  $\beta$ -cells of the pancreas. The presence of 11 $\beta$ -HSD1 in pancreatic islets makes these cells more sensitive to glucocorticoids (Davani et al., 2000). In addition, 11 $\beta$ -HSD1 mediated increased glucocorticoid sensitivity in adipose tissue may play a role in the etiology of obesity and metabolic syndrome. Support for this role of 11 $\beta$ -HSD1 was found by Mazusaki and colleagues, who observed that mice overexpressing 11 $\beta$ -HSD1 in adipose tissue had increased glucocorticoid levels and fat deposition, insulin-resistant diabetes and hyperlipidemia (Masuzaki et al., 2001). GR levels are themselves negatively regulated by glucocorticoids, in line with long-term treatment resulting in a decreased physiological response (Dong et al., 1988; Okret et al., 1991). Reduced sensitivity may also be a result of mutations that in some way inactivate the GR (Ruiz et al., 2001). Finally, in line with GR cross-talk with other signaling pathways often is mutual, conditions of increased activity of these pathways may negatively influence glucocorticoid sensitivity. For instance, increased AP-1 activity correlating with decreased GR sensitivity has been observed in subjects suffering from glucocorticoid resistant asthma (Adcock and Lane, 2003). In addition, increased MAPK activity has been suggested to decrease glucocorticoid sensitivity by phosphorylating the GR (Li et al., 2004; Tsitoura and Rothman, 2004).

### **Glucocorticoid therapeutic effects**

Synthetic glucocorticoids such as dexamethasone prednisolone and triamcinolone acetonide have long been the primary treatment of inflammatory and immune disorders. Prolonged treatment, however, is accompanied with side effects including osteoporosis, type 2 diabetes, growth retardation, impaired wound healing and hypertension, and therefore limits the use of glucocorticoids (Schäcke et al., 2002). The conceptual view is that the majority of side effects are mediated through GR binding to and activation of GRE containing genes involved in metabolic pathways, whereas the antiinflammatory and immunosuppressive effects of glucocorticoids are mediated via GRE independent mechanisms, such as protein-protein interactions. In support of this idea, the GRdim mice (discussed above) are able to repress an inflammatory challenge in a glucocorticoid dependent manner (Reichardt et al., 2001).

Given that both NF- $\kappa$ B and AP-1 have been shown to be crucial for the induction of genes involved in inflammation and the well-recognized GRE independent glucocorticoid inhibition of these factors, support for GRE independent antiinflammatory effect is provided ((De Bosscher et al., 2003) and references therein). So far, reduction of the unwanted effects is partially achieved by local administration of glucocorticoids, e.g. inhalation and dermal application. Another way to reduce the side effects would be to develop synthetic glucocorticoid hormones that induce a receptor conformation that is able to dissociate GR transactivation from transrepression. As indicated by the studies employing the GRdim mice, such compounds, termed 'dissociated' glucocorticoids, should still be able to efficiently inhibit inflammation. To date, however, such dissociated compounds have met with little success in vivo (Belvisi et al., 2001). An additional step to achieve more specific glucocorticoid effects would be to further dissociate GR cross-talk with NF- $\kappa$ B and AP-1, respectively, since they may be differentially important for various biological processes.

### **TRANSCRIPTION FACTOR NF- $\kappa$ B**

The NF- $\kappa$ B regulates many cellular functions such as inflammation, immune response, apoptosis, oncogenesis and differentiation (Ghosh and Karin, 2002; Karin et al., 2002). Association between normal growth and NF- $\kappa$ B activation has also been noted in many cells and tissues ((Chen et al., 2001) and references therein). NF- $\kappa$ B is an inducible dimeric transcription factor, most commonly described as a heterodimer between p65 (RelA) and p50 (NF- $\kappa$ B1). There are five members in the mammalian NF- $\kappa$ B family, which includes p65, RelB, c-Rel, p50 (its precursor p105) and p52 (NF- $\kappa$ B2, its precursor p100). All members of the NF- $\kappa$ B proteins share a highly conserved 300 amino acid long N-terminal Rel homology domain (RHD). This domain is responsible for DNA binding, dimerization with the different Rel family members and association with the I $\kappa$ B inhibitory proteins. Three of the members, p65, RelB and c-Rel contain transcriptional activation domains (TADs). The I $\kappa$ B family in turn, consists of I $\kappa$ B $\alpha$ , I $\kappa$ B $\beta$ , I $\kappa$ B $\epsilon$ , I $\kappa$ B $\gamma$ , Bcl-3 and the precursors proteins p105 and p100. In unstimulated

cells NF- $\kappa$ B resides in the cytoplasm bound to I $\kappa$ B. The crystallographic structure of I $\kappa$ B $\alpha$  bound to the p65/p50 dimer revealed that the I $\kappa$ B $\alpha$  mask only the nuclear localization signal sequence (NLS) of p65, whereas the NLS of p50 remains exposed. The accessible NLS on p50 together with the presence of nuclear export signals (NES) on I $\kappa$ B $\alpha$  and p65 results in constant shuttling between the nucleus and the cytoplasm, however, the majority of NF- $\kappa$ B/I $\kappa$ B $\alpha$  complexes at a given time point is localized in the cytosol ((Hayden and Ghosh, 2004) and references therein). A large number of extracellular stimuli activate NF- $\kappa$ B, including the cytokines interleukin-1 (IL-1), tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), lipopolysaccharide (LPS), phorbol esters and virus infection. NF- $\kappa$ B activation involves phosphorylation of I $\kappa$ B $\alpha$  at serines 32 and 36 by a high molecular weight I $\kappa$ B kinase (IKK) complex. This sequential phosphorylation cascade subsequently leads to ubiquitinylation of I $\kappa$ B $\alpha$  and targets its degradation by the 26S proteasome, allowing NF- $\kappa$ B to translocate into the nucleus and modulate gene transcription through interaction with specific NF- $\kappa$ B binding sites in promoter regions of target genes. Mutation of serines 32 and 36 of the I $\kappa$ B $\alpha$  protein results in a stable (superrepressive) form of I $\kappa$ B $\alpha$  that efficiently prevents NF- $\kappa$ B activation (Brown et al., 1995). Although degradation of I $\kappa$ B $\alpha$  is sufficient to cause NF- $\kappa$ B translocation into the nucleus, other events can affect NF- $\kappa$ B's ability to activate transcription. For example, posttranslational modification such as phosphorylation of the NF- $\kappa$ B proteins may modulate their transcriptional activity (Karin and Ben-Neriah, 2000). In addition to phosphorylation of I $\kappa$ B $\alpha$ , the IKK complex has been shown to phosphorylate p65 at serine 536, an event that takes place in the cytoplasm (Sakurai et al., 1999). The functional role for this phosphorylation, however, awaits further clarification. An additional cytoplasmic phosphorylation mechanism of p65 has been reported. In this case an interaction with protein kinase A catalytic subunit (PKAc), I $\kappa$ B and p65 was identified suggesting a PKAc mediated phosphorylation of p65 at serine 276 that correlated with increased transcriptional activity (Zhong et al., 1997). More recently, the serine 276 residue of p65 has also been reported to be phosphorylated by the mitogen- and stress-activated protein kinase-1 (MSK1) (Vermeulen et al., 2003b). This kinase, recently identified and characterized, resides in the nucleus and is activated by stimulators acting via extracellular signal-regulated kinase (ERK) and/or via p38 MAPK pathways (Deak et al., 1998). Thus, a single residue of p65 is targeted by two



kinases in distinct cellular compartments. Interestingly, phosphorylation of serine 276 is suggested to promote efficient recruitment of the coactivator CBP, which provides an explanation for the increased transcriptional activity of NF- $\kappa$ B following phosphorylation of p65 at this site (Vermeulen et al., 2003b; Zhong et al., 1998). Finally, a negative autoregulatory mechanism ensures balanced NF- $\kappa$ B activity. The promoter of I $\kappa$ B $\alpha$  contains binding sites for NF- $\kappa$ B allowing it to induce expression of its own inhibitor (Algarde et al., 1999).

## **TRANSCRIPTION FACTOR AP-1**

AP-1 transcription factors are either homo- or heterodimers of proteins belonging to the Jun (c-Jun, JunB and JunD) and Fos (c-Fos, FosB, Fra-1 and Fra-2) subfamilies. In addition Jun and Fos can heterodimerize with other proteins, such as activating transcription factor (ATF) and Maf families (Angel and Karin, 1991; Mechta-Grigoriou et al., 2001; Shaulian and Karin, 2002). The AP-1 dimer constitutes a transcription factor that is involved in a diverse set of cellular functions such as proliferation, differentiation and apoptosis. Since promoter regions of several proinflammatory cytokines contain binding sites for AP-1, similar to NF- $\kappa$ B (see above), a role for AP-1 in mediating inflammatory responses is supported (De Bosscher et al., 2003; Sadikot et al., 2004). Similar to NF- $\kappa$ B, the activity of AP-1 can be stimulated by various extracellular signals, including cytokines, growth factors, phorbol ester and cellular stress. These signals activate the MAPK pathways, which consist of three families of protein kinases, extracellular signal-regulated kinases (ERKs), c-Jun N-terminal kinases (JNKs) and p38 kinases (Firestein and Manning, 1999). The most commonly described AP-1 complex consists of c-Jun and c-Fos heterodimer binds to AP-1 DNA recognition elements, also known as TREs (TPA response elements) in promoter regions of target genes and modulate transcription. All three families of protein kinases, ERK, JNK and p38, are able to contribute to upregulation of c-Fos expression, while transcription of c-Jun primarily is controlled by itself via TREs located in the c-Jun promoter in a positive autoregulatory loop. In addition, phosphorylation of c-Jun by JNK on serines 63 and 73 has been shown to be

an important route for activation. Phosphorylation increases the stability of the protein to a certain extent, and importantly, it increases c-Jun's transcriptional activity (Karin et al., 1997; Shaulian and Karin, 2001). Similar to phosphorylation of p65 on serine 276, this phosphorylation step of c-Jun is required to recruit the transcriptional coactivator CBP (Bannister et al., 1995). Thus, the c-Jun gene is both a target for the MAPK cascade and encodes a protein that mediates MAPK signaling into a transcriptional response.

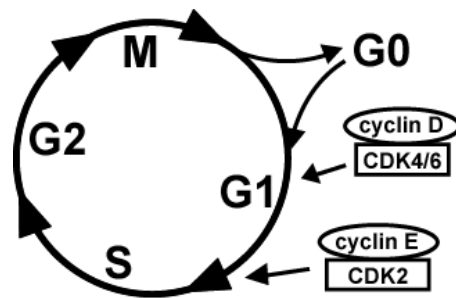
## **GLUCOCORTICOID INTERFERENCE WITH MAPKs**

In contrast to the direct signaling transduction pathway exemplified by nuclear receptor regulation of gene expression, MAPK signaling provides an indirect route from cell stimulus to transcriptional regulation. MAPKs are components of a three-kinase regulatory phosphorylation cascade. In this case MAPKs are phosphorylated by MAPK kinases (MEKs or MKKs), which in turn are phosphorylated by MKK kinases (MEKKs or MKKKs) (Pearson et al., 2001). Several distinct MAPK cascades have been identified, and the three most common ones are the extracellular signal-regulated kinase (ERK), the c-Jun N-terminal kinase (JNK) and the p38 cascades. MAPK pathways can be activated by a plethora of extracellular stimuli such as growth factors, cytokines, cellular stress, phorbol esters and lipopolysaccharide. Activation of MAPKs by these stimuli controls gene expression (Garrington and Johnson, 1999). More recently, an increased attention has been given the role of MAPKs' during inflammation. The fact that many of the proinflammatory signals activate MAPKs signaling, suggests a role for MAPK in inflammatory response (Firestein and Manning, 1999; Karin, 2004). The duration and amplitude of MAPK activation is a balance of activating signal and inactivation mechanisms. Negative regulation of e.g. ERK is, at least in part, through dephosphorylation of ERK by dual-specificity phosphatases, e.g. MAPK phosphatase-1 (MKP-1) (Pearson et al., 2001). This phosphatase also dephosphorylates other MAPKs, e.g. p38 and JNK (Theodosiou and Ashworth, 2002). Interestingly, recent reports have shown that glucocorticoids induce MKP-1 expression, which correlates with a decreased ERK activity (Engelbrecht et al., 2003; Kassel et al., 2001; Wu et al., 2004b). Based on transfection experiments using a

MKP-1 promoter gene reporter and GR mutants unable to activate gene transcription via GREs, Kassel and colleagues speculate that MKP-1 is upregulated via GREs located in the promoter. The function of these putative GREs, however, remains to be characterized. Glucocorticoid interference of MAPK signaling is not limited to ERK inhibition. For instance, glucocorticoids have been suggested to inhibit the activity of p38 (Lasa et al., 2001). Moreover, it has been reported that glucocorticoids can interfere with MAPK JNK signaling (Bruna et al., 2003; Caelles et al., 1997). Here, a direct GR interaction with JNK, via a hormone regulated JNK docking site in the GR LBD, is suggested for inhibition of JNK activation. Interestingly, the glucocorticoid inhibition of MAPKs is mutual, i.e. MAPK can inhibit the activity of GR. For example, both JNK and ERK mediated phosphorylation of GR has been shown to result in reduced GR mediated transcriptional activation (Li et al., 2004; Rogatsky et al., 1998). Given that many of the proinflammatory signals activate MAPKs signaling, glucocorticoid inhibition of MAPKs may contribute to the antiinflammatory effect exerted by glucocorticoids. However, its relative contribution to direct GR cross-talk with NF- $\kappa$ B and AP-1 is not clear.

## **CELL CYCLE**

Central to the process of mitosis (division of one cell into two daughter cells) is DNA replication which is under the tight regulation of the cell cycle. One mitosis equals one round in the cell cycle, which in turn is divided into four active phases (G1, S, G2 and M) as well as one inactive phase (G0) (Fig.4). The cell enters the cell cycle in the G1-phase, which is the only phase dependent on growth factors. Subsequently, DNA synthesis takes place in the S-phase followed by the G2-phase during which the cell completes the DNA synthesis and prepares for mitosis. Finally, the M-phase completes the cell cycle.



**Figure 4.** Schematic illustration of the cell cycle and cyclin-CDK complexes involved in G1-S transition.

The transition from one phase to another occurs in an orderly fashion and is regulated by different cellular proteins. Key regulatory proteins are the cyclin-dependent kinases (CDKs), a family of kinases that are activated at specific points of the cell cycle. Direct binding of CDK proteins to cyclins, a family of regulatory proteins, is a prerequisite for its activity. While CDK protein levels remain stable during the cell cycle, the expression pattern of cyclins is periodic and thereby restricts the activity of each CDK during the cell cycle. Different cyclins are required at different phases of the cell cycle. Briefly, CDK4 and CDK6 form complexes with cyclin D proteins and are essential for G1 entry. Progression from G1 into S-phase is regulated by cyclin E-CDK2 complex and during the S-phase a complex of cyclin A-CDK2 is required. Subsequently, during G2 and M-phases cyclin A complexes with CDK1 to promote entry into M. Mitosis is further regulated by cyclin B-CDK1 complex (Vermeulen et al., 2003a). In addition to activation of CDKs by cyclin interaction, the CDK activity can be hindered by cell cycle inhibitory proteins termed CDK inhibitors (CKIs), via direct protein-protein interactions. There exist two families of CKIs, the INK4 family and the Cip/Kip family. The INK4 family consists of p15<sup>INK4b</sup>, p16<sup>INK4a</sup>, p18<sup>INK4c</sup> and p19<sup>INK4d</sup> and the second family includes p21<sup>Cip1</sup>, p27<sup>Cip2</sup> and p57<sup>Kip2</sup>. Although the latter family binds to and inhibits cyclin D-, E- and A-dependent kinases *in vitro*, their main CDK inhibitory function seems to be mediated through cyclin E- and A-dependent CDK2 (Sherr and Roberts, 1999).

### **Glucocorticoids and cell proliferation**

Despite the well-recognized antiproliferative effect by glucocorticoids, the molecular mechanism has remained largely unclear. Although altered expression of genes involved in the cell cycle following glucocorticoid treatment have been observed, few examples of primary target genes have been demonstrated that are directly responsible for the antiproliferative effect. The antiproliferative effect is of clinical importance, since several clinical adverse effects induced by long-term glucocorticoid treatment are associated with cell growth inhibition, e.g. skin atrophy, impaired wound healing and osteoporosis (Schäcke et al., 2002). Although inhibition of cell growth by glucocorticoids is most often an unwanted effect, there are physiological conditions where this effect may be therapeutic. For example, the glucocorticoid antiproliferative effect is utilized in tumor therapy, e.g. breast cancer ((Schäcke et al., 2002) and references therein).

Glucocorticoids have been shown to inhibit cell proliferation in a variety of cell types, e.g. hepatoma cells, fibroblasts and osteoblasts, with an inhibition occurring in G1-phase (Corroyer et al., 1997; Sanchez et al., 1993; Smith et al., 2000). Further, ectopic expression and activation of GR in human cells lacking functional GR, e.g. U2OS, SAOS2 and HEK293, result in G1 cell cycle arrest (Bladh et al., 2005; Rogatsky et al., 1997). As outlined in the previous section, proteins that play important roles during the cell cycle include the cyclins that are responsible for regulating the cell cycle and the CKIs, which in turn regulate the activity of the cyclins. In line with this, altered expression patterns of these proteins involved in cell cycle regulation have been detected in association with glucocorticoid repressed cell growth (Rogatsky et al., 1997). In human osteosarcoma U2OS cells, a GR mutant (N-terminal deletion) lacking transactivation properties still inhibited cell proliferation, indicating that repression of transcription may be involved in this antiproliferative effect. Further support for involvement of a GR mediated transrepressive mechanism was provided by the finding that both the wild type GR and the GR mutant repressed expression of cell cycle activating proteins such as CDK4, CDK6 as well as their regulatory partner cyclin D3. In contrast to U2OS cells, glucocorticoid mediated inhibition of cell proliferation in SAOS2 cells was reported to depend on the transactivation properties of the GR. Here, it was shown that glucocorticoid treated cells expressed higher levels of cell cycle

inhibitory proteins, such as p21<sup>Cip1</sup> and p27<sup>Kip1</sup>, which correlated to a glucocorticoid mediated inhibition of cell proliferation. In addition, inhibition of cell proliferation by hormone activated GR in HeLa cells correlates with upregulation of the cell cycle inhibitory protein p57<sup>Kip2</sup> (Samuelsson et al., 1999). Later on, we demonstrated a functional GRE in the p57<sup>Kip2</sup> promoter, indicating that upregulation of p57<sup>Kip2</sup> by glucocorticoids is through a GRE dependent mechanism, and thus, further supports that transcriptional activation by GR is involved in the antiproliferative effect (Alheim et al., 2003). In summary, two distinct regulatory mechanisms of GR mediated cell cycle arrest are indicated, one involves transcriptional repression of G1 cyclins and CDKs and the other involves transcriptional activation of CDI expression. The importance for each mechanism, transactivation versus transrepression via the activated GR, seems to be context dependent. However, the determinants responsible for a given mechanism to be active in a certain context remain to be clarified.

With regard to the mechanism relating to GR mediated transcriptional repression, inhibition of transcription factor complexes such as AP-1 and NF- $\kappa$ B may be involved in glucocorticoid mediated antiproliferative effect. Several lines of evidence implicate AP-1 involvement in cellular proliferation. For example, growth factors induce expression of c-Fos and c-Jun, cells deficient in c-Jun expression exhibit significantly altered cell growth properties and c-Jun has been shown to be required for G1- to S-phase transition (Schreiber et al., 1999; Shaulian and Karin, 2002; Wisdom et al., 1999). Notably, one of the AP-1 subunits, JunB, has been suggested to negatively regulate proliferation, in contrast to AP-1's positive effects on cell proliferation that are most frequently reported (Shaulian and Karin, 2002). Taken together, the importance of AP-1 as a modulator of cell proliferation is undisputed, and thus, GR inhibition of AP-1 is an attractive model for glucocorticoid-induced inhibition of cell proliferation. This model, however, has not yet been confirmed.

Considering that the transcription factor NF- $\kappa$ B has been extensively studied during the years, remarkably limited amount of information is available regarding the possible involvement of NF- $\kappa$ B in cell cycle regulation. Early observations in mouse fibroblasts showed that NF- $\kappa$ B activity was increased during G0 to G1 transition suggesting a role

in cell cycle regulation (Baldwin et al., 1991). Subsequently, it has been demonstrated that inhibition of NF- $\kappa$ B cause impairment of cell cycle progression in glioma cells and retard cell cycle G1 to S transition in HeLa cells (Kaltschmidt et al., 1999; Otsuka et al., 1999). The increasing amount of evidence infers NF- $\kappa$ B in cell cycle regulation and, in analogy with AP-1, the documented glucocorticoid mediated repression of NF- $\kappa$ B activity can be an important mechanism contributing to the antiproliferative effect of glucocorticoids. Indeed, a recent report showed that suppression of GR levels leads to an increased activity of NF- $\kappa$ B, which correlates with an enhanced cell proliferation in murine macrophage RAW264.7 cells (Zhu et al., 2004). In line with this, we demonstrate a correlation between GR repression of NF- $\kappa$ B (and not AP-1) and the antiproliferative effect (Bladh et al., 2005).

As described earlier, the cyclins are important regulators of cell cycle progression. In association with CDKs, the cyclins promote cell cycle progression. In fact, recent research on cyclin D and E revealed positive correlations between the overexpression of cyclins and the pathogenesis of breast cancer ((Wu and Kral, 2005) and references therein). In addition, transgenic mice that overexpress cyclin D1 in mammary glands result in abnormal mammary cell proliferation including breast cancer (Wang et al., 1994). In their capacity as positive regulators of cell proliferation, cyclins may be a downstream target of glucocorticoid antiproliferative action. In this respect, glucocorticoid mediated downregulation of cyclins probably occur via GR negative cross-talk with signaling pathways regulating cyclin expression. Interestingly, the promoter region of cyclin D1 contains functional binding sites for both AP-1 and NF- $\kappa$ B (Guttridge et al., 1999; Hinz et al., 1999; Shaulian and Karin, 2001; Wisdom et al., 1999). The indication that both AP-1 and NF- $\kappa$ B have the ability to control the levels of cyclin D1, suggests cyclin D1 as an important downstream target gene involved in the antiproliferative effect of glucocorticoids. In summary, the antiproliferative effects of glucocorticoids can be mediated by GR actions on several levels, including direct transcriptional activation and interference with other signaling transduction pathways.

## AIMS OF THE STUDY

GR mediates the action of glucocorticoids, which have well-recognized properties that include antiinflammatory and antiproliferative effects. This thesis focuses on GR interference with two signaling transduction pathways, NF- $\kappa$ B and AP-1, which are involved in both inflammatory responses and cell proliferation. While it is known that GR cross-talk with these two pathways, their individual contribution to glucocorticoid antiinflammatory and antiproliferative effects is poorly understood. To further study the molecular mechanisms and functional role of this cross-talk, the following aims were stated:

- To investigate the possibility to separate GR's cross-talk with NF- $\kappa$ B and AP-1 signaling transduction pathways (Paper I).
- To investigate molecular mechanisms involved in glucocorticoid mediated repression of NF- $\kappa$ B (Paper II).
- To investigate the role of NF- $\kappa$ B and AP-1 in GR mediated inhibition of cell proliferation (Paper III).
- To identify and characterize potential GREs in the cyclin dependent kinase inhibitor p57<sup>Kip2</sup>, a downstream target of GR that is involved in inhibition of cell proliferation (Paper IV).



## RESULTS AND DISCUSSION

### Paper I-II

The GR negative cross-talk with NF- $\kappa$ B and AP-1 signaling transduction pathways has been known for several years and is believed to form the basis for the glucocorticoid antiinflammatory effect. A number of mechanisms have been suggested to explain GR interference with the two pathways (see introduction in the thesis), where some suggest that similar mechanism could account for inhibition of both NF- $\kappa$ B and AP-1 by GR. The glucocorticoids have well-recognized antiinflammatory and immunosuppressive effects and are widely used in the clinic to treat chronic disorders such as asthma rheumatoid arthritis (Schäcke et al., 2002). However, severe side effects accompanying long-term treatment of glucocorticoids limit their use, and hence, there is an interest to develop novel compounds with reduced number of side effects, while the antiinflammatory properties are retained. Based on the hypothesis that transactivation of genes by GR through a GRE dependent mechanism is dispensable for the antiinflammatory effect, there is an interest in so-called dissociated glucocorticoids. These compounds display poor transactivation properties, while still being functional with regard to inhibition of inflammation (Vayssiere et al., 1997). So far, however, such compounds have met with little success in vivo (Belvisi et al., 2001). An additional step to achieve more specific effects would be to further dissociate GR cross-talk with NF- $\kappa$ B and AP-1, respectively, since they may be differentially important for various biological processes. The possibility to separate GR cross-talk with NF- $\kappa$ B and AP-1 and cross-talk mechanisms is investigated in paper I and II.

By the use of GR DBD mutants we were able to separate GR cross-talk with NF- $\kappa$ B and AP-1 signaling transduction pathways. At the time when the work of Paper I was initiated, the involvement of the DBD region in GR cross-talk with NF- $\kappa$ B and AP-1 was well known. In addition, some reports indicated that the N-terminal and C-terminal zinc-finger of the GR DBD could be important for AP-1 and NF- $\kappa$ B repression, respectively (Heck et al., 1994; Lidén et al., 1997). In line with this, we

identified a C-terminal zinc-finger mutant, GR<sub>R488Q</sub>, which in addition to containing a known impaired ability to inhibit NF-κB activity, had a preserved capacity to repress AP-1 dependent signaling. This finding was further substantiated by the fact that endogenous genes preferentially regulated by NF-κB and AP-1, respectively, were identified to respond similar to the corresponding reporter genes. The gene expression profiling revealed genes differentially regulated by GR<sub>R488Q</sub> versus wild type GR at 2 h of treatment mainly to be involved in control of transcription and cell growth. At 8 h, no such distinction could be seen. In search for a possible explanation of the GR<sub>R488Q</sub> mutant's impaired function to repress NF-κB activity, we introduced another GR DBD mutant (GR<sub>LS7</sub>) in our experiments, a GR mutant with known capacity to repress both NF-κB and AP-1, while being a poor transactivator through GREs (Lidén et al., 1997; Yang-Yen et al., 1990). Indeed, both NF-κB and AP-1 activity were repressed by GR<sub>LS7</sub>, thus, these results suggested that repression of NF-κB activity does not involve activation of GRE regulated genes. Since a physical interaction between GR and p65 (RelA) has been described to be involved in the GR mediated repression of NF-κB, we next investigated if the R488Q mutation affected the receptor's ability to physically interact with p65 (Caldenhoven et al., 1995). Similar to GR<sub>wt</sub>, an interaction between GR<sub>R488Q</sub> and p65 was detected, demonstrating that a physical interaction is not sufficient for a functional GR mediated repression of NF-κB. Possibly the mutant is not able to recruit or interfere with other factor(s) necessary for a functional inhibition of NF-κB. Such interference has been suggested to occur at the ICAM-1 and IL-8 promoters leading to inhibition of polymerase II phosphorylation (Nissen and Yamamoto, 2000). However, the factor responsible for this effect remains to be identified.

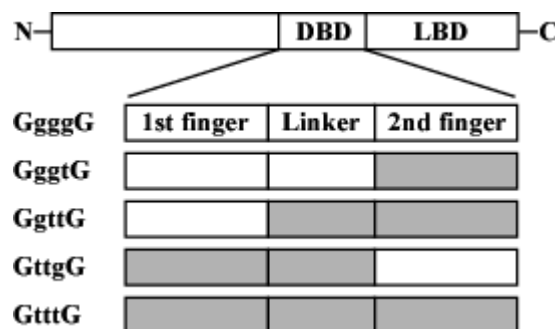
According to these results (see above), neither a physical interaction with NF-κB nor upregulation of gene expression via classical GREs, seem to play critical roles in GR cross-talk with NF-κB in HEK293 cells. In the search for an explanation to GR<sub>R488Q</sub>'s failure to inhibit NF-κB, we next tested the ability of GR<sub>R488Q</sub> to repress NF-κB following treatment of different NF-κB activators. In contrast to what we observed with phorbol ester (TPA) activation of NF-κB, GR<sub>R488Q</sub> could inhibit NF-κB activity following TNFα stimulation. This indicated that the R488Q mutation impaired the

repressive property of the GR during defined but not all conditions, and that GR mediated repression of activated NF- $\kappa$ B required separate functions of the GR, functions that were dependent on the signaling pathway employed to activate NF- $\kappa$ B. Based on reports showing that the GR interferes with MAPK ERK signaling and that TPA activates ERK and not p38 in HEK293 cells, the role of MAPK ERK signaling for GR mediated repression was investigated (Grab et al., 2004; Rider et al., 1996). First the ability of GR<sub>wt</sub> and GR<sub>R488Q</sub> to inhibit TPA activated ERK was tested, which revealed that GR<sub>wt</sub> but not GR<sub>R488Q</sub> inhibited ERK activation. This suggested to us that ERK repression by GR is necessary for glucocorticoid-mediated repression of NF- $\kappa$ B activity. By using a MEK inhibitor (U0126) to inhibit ERK activation, we could test this hypothesis. It was demonstrated that hormone activated GR<sub>R488Q</sub> in combination with ERK inhibition, restored the mutant's ability to repress TPA activated NF- $\kappa$ B. ERK inhibition had no effect on either TPA or TNF $\alpha$  to activate NF- $\kappa$ B. Thus, ERK inhibition seemed to be important for glucocorticoid mediated repression of NF- $\kappa$ B, which was further emphasized by the fact that TPA but not TNF $\alpha$  activated ERK in HEK293 cells. In search for a possible mechanistic explanation to these results, we investigated a phosphatase, MAPK phosphatase-1 (MKP-1), known to deactivate MAPKs via dephosphorylation (Theodosiou and Ashworth, 2002). In addition, previous reports had shown a glucocorticoid-mediated inhibition of ERK concomitant with an upregulation of MKP-1 protein levels, an upregulation suggested to be GRE dependent (Engelbrecht et al., 2003; Kassel et al., 2001). However, to date no functional GREs have been demonstrated in the MKP-1 promoter. In a series of experiments, we could demonstrate that ectopic expression of MKP-1 in HEK293 cells resulted in inactivation of TPA stimulated ERK and restored GR<sub>R488Q</sub>'s ability to repress TPA stimulated NF- $\kappa$ B, and further, GR<sub>wt</sub> but not GR<sub>R488Q</sub> induced MKP-1 expression. Together these results was in line with a hypothesis that a glucocorticoid mediated upregulation of MKP-1 was necessary for GR to repress TPA stimulated NF- $\kappa$ B activity. According to this idea, inhibition of MKP-1 by some means should impair GR's ability to repress TPA activated NF- $\kappa$ B. In order to test this hypothesis, we silenced the expression of MKP-1 by RNA interference and could show that the ability of GR<sub>wt</sub> to inhibit TPA stimulated NF- $\kappa$ B was compromised. In summary, paper I identifies a transactivation deficient GR mutant that is able to separate between NF- $\kappa$ B

(impaired) and AP-1 (maintained) repression, which demonstrates that different receptor surfaces or functions are involved in repression of NF- $\kappa$ B and AP-1, respectively. Using this ‘loss-of-function’ mutant, the requirement of ERK inactivation in conjunction with GR repression of NF- $\kappa$ B is revealed in paper II. Furthermore, glucocorticoid mediated upregulation of MKP-1 is identified as a possible mechanism by which the GR deactivates ERK.

### Paper III-IV

Glucocorticoids exert an antiproliferative effect in most cell types. However, the molecular mechanism is still largely unclear. As described in this thesis, glucocorticoids act by binding to GR, which then stimulates or inhibits transcription of target genes. In relation to the antiproliferative effect, GR may either repress expression of genes that stimulate cell proliferation (paper III) or alternatively enhance the expression of genes that exert an antiproliferative effect (paper IV). Thus, with emphasis on cell proliferation, paper III and IV demonstrates the complexity of glucocorticoid signaling.



**Figure 5.** Schematic representation of the different GR mutants containing chimeric GR/TR $\beta$  DBDs (paper III).

A novel finding is made in paper III, where glucocorticoid inhibition of cell proliferation involves GR mediated repression of NF- $\kappa$ B. Using GR DBD mutants unable to transactivate through GREs and with different efficacy to repress NF- $\kappa$ B and

AP-1 activity, a good correlation between the antiproliferative effect and the ability of glucocorticoids to repress NF- $\kappa$ B activity was observed (Fig.5). The antiproliferative domain of the GR was localized to the C-terminal zinc-finger, which was the same region shown to be required for NF- $\kappa$ B repression. Although further testing with additional mutants are required, the results indicated that GR-AP-1 cross-reactivity involves the linker region of the GR DBD, since maintaining the GR linker region (GggtG→GggtG) sustained approximately 50% of the AP-1 repressive activity as compared with the GR<sub>wt</sub>. AP-1 repressing activity was also present in the N-terminal zinc-finger of TR $\beta$  DBD, since replacing the GR N-terminal zinc-finger with corresponding zinc-finger of the TR $\beta$  (GggtG→GtttG) restored 50-60% of the AP-1 repressing activity of GR<sub>wt</sub>. Again (see paper I), we observed that GR DBD mutants could separate between repression of NF- $\kappa$ B (impaired) and AP-1 (maintained), further highlighting the DBD of the GR as an important region for this cross-talk. In addition, these results are in line with reports from Jurkat, CEM-C7 T cells, and U2OS cells in which it has been demonstrated that repression rather than activation of genes via GR binding to GREs is important for the antiproliferative response (Helmberg et al., 1995; Rogatsky et al., 1997; Thompson et al., 1992). In our case, this conclusion was based on the results obtained using the GtttG mutant, which lacked the ability to transactivate through GREs, but maintained the transrepressive and antiproliferative activity. However, the antiproliferative mechanism may differ in different cell lines, e.g. in HeLa, S49 and SAOS2 cells the transactivation function of the GR is required for the antiproliferative effect (Chapman et al., 1996; Rogatsky et al., 1997; Samuelsson et al., 1999).

In HeLa cells, the cyclin-dependent kinase inhibitor p57<sup>Kip2</sup> is suggested to be involved in the glucocorticoid induced antiproliferative effect. It was demonstrated that glucocorticoid induction of p57<sup>Kip2</sup> expression resulted in a G1-phase accumulation of the cells. The p57<sup>Kip2</sup> induction by glucocorticoids was a direct response as no *de novo* protein synthesis was required, suggesting a direct interaction of the GR with regulatory elements in the p57<sup>Kip2</sup> promoter. Therefore, in paper IV, we aimed at identifying putative GREs in the p57<sup>Kip2</sup> promoter. Using different deletion constructs of the p57<sup>Kip2</sup> promoter, ranging from just over 100 bases up to 6.3 kilo bases (kb)

long, a 40 bases long sequence containing a putative GRE was identified, which was located 5076-5062 bases upstream of the transcription start site. Fused to a reporter gene, it was demonstrated that this 40 bases long sequence was sufficient to confer glucocorticoid activation. Moreover, mutation of the GRE abolished glucocorticoid induction of the reporter gene and in electrophoresis mobility shift assays it was demonstrated that the p57<sup>Kip2</sup> GRE could compete with a well-known GRE for GR binding. In summary, these results demonstrate that glucocorticoid induced p57<sup>Kip2</sup> expression is mediated via a GRE in the promoter of p57<sup>Kip2</sup>. The fact that this GRE is conserved within the mouse genome, the biological importance of this GRE is further strengthened.

In contrast to HeLa cells, upregulation of p57<sup>Kip2</sup> does not seem to be necessary for the glucocorticoid antiproliferative effect in HEK293 cells. Rather, an indication for the involvement of NF- $\kappa$ B in the glucocorticoid mediated antiproliferative effect was observed (see above), which was further substantiated from gene expression profiling studies in HEK293 cells containing two GR mutants, GttgG and GtttG, respectively. These mutants lack the ability to transactivate GRE controlled genes, of which only one can repress NF- $\kappa$ B (GttgG), whereas both can repress AP-1. In line with being transactivation deficient and that glucocorticoid induction of p57<sup>Kip2</sup> expression occurs via a GRE dependent mechanism, these GR mutants did not display an upregulation of p57<sup>Kip2</sup> expression. Unexpectedly, however, among the limited number of genes that were regulated in the microarray following glucocorticoid treatment, 18 of 21 genes in GttgG and 6 of 7 genes in GtttG were upregulated. One of the genes upregulated was I $\kappa$ B $\alpha$ , which was induced in GttgG- but not in GtttG expressing cells, a finding that also was confirmed at the protein level. This was particularly interesting, since the GttgG was able to repress NF- $\kappa$ B and, importantly, executed an antiproliferative effect. Further investigation of the role of I $\kappa$ B $\alpha$  induction revealed that selective expression of I $\kappa$ B $\alpha$  was sufficient to both inhibit cell proliferation and reduce NF- $\kappa$ B activity, in line with NF- $\kappa$ B being important for cell cycle progression. Collectively, these results suggest that glucocorticoid mediated repression of NF- $\kappa$ B activity is an important mechanism contributing to the antiproliferative effect. However, it cannot be excluded that other mechanisms can be involved, such as GR mediated posttranslational

modifications of proteins involved in cell cycle control. Also, genes regulated less than the applied filter for selecting regulated genes may be involved and/or other genes that are not included on the array could possibly be involved in the glucocorticoid mediated antiproliferative effect. The array holds about 8,500 genes of the approximately 38,000 genes present in the human genome. Together with the results in paper IV, these findings demonstrate the complexity of glucocorticoid signaling, where the antiproliferative effects of glucocorticoids can be mediated by GR actions on several levels, including interference with other signaling transduction pathways (paper III) and direct transcriptional activation (paper IV).

## CONCLUSIONS AND FUTURE PERSPECTIVES

The investigations presented in this thesis established the following findings:

- GR cross-talk with NF- $\kappa$ B and AP-1 signaling transduction pathways can be separated by introducing mutations in the GR DBD. Furthermore, the GR<sub>R488Q</sub> (and GtttG) discriminating activity between NF- $\kappa$ B and AP-1 suggests that different receptor surfaces or mechanisms are involved in repression of NF- $\kappa$ B and AP-1, respectively. Endogenous genes preferentially repressed by GR interference with NF- $\kappa$ B activity were identified. The genes differentially regulated by the GR<sub>R488Q</sub> mutant versus the wild type GR contribute to a variety of biological functions.
- Using the ‘loss-of-function’ mutant (GR<sub>R488Q</sub>), inhibition of activated extracellular signal-regulated kinase (ERK) was identified as an important determinant for a functional GR mediated repression of NF- $\kappa$ B. Induction of MKP-1, an endogenous inhibitor of ERK, was identified as a candidate glucocorticoid target gene responsible for inactivating stimulated ERK, which was necessary for a functional glucocorticoid mediated repression of NF- $\kappa$ B during conditions where ERK was activated.
- Identification of glucocorticoid mediated inhibition of NF- $\kappa$ B as an important mechanism in the antiproliferative response to glucocorticoids. Glucocorticoid induction of the NF- $\kappa$ B inhibitor, I $\kappa$ B $\alpha$ , was demonstrated to be an important factor mediating the antiproliferative response to glucocorticoids.
- A functional glucocorticoid response element (GRE) is present 5 kb upstream of the transcriptional start site in the human p57<sup>Kip2</sup> promoter. The GRE was shown to be well conserved in the mouse, both regarding sequence similarity and function, indicating a high biological relevance for this element.



Glucocorticoid hormones, e.g. cortisol and cortisol analogues, are potent anti-inflammatory and immunosuppressive agents and are widely used in the clinic for treatment of e.g. rheumatoid arthritis and asthma. The intracellular protein, GR, which is present in most tissues, mediates the effects observed following glucocorticoid treatment. Long-term glucocorticoid treatment, however, is associated with a number of side effects such as osteoporosis, muscle wasting, growth inhibition, type 2 diabetes and hypertension. Although the molecular mechanisms involved in activation and repression of glucocorticoid target genes are quite well known, the signaling pathways responsible for the different side effects are poorly understood. One way to reduce unwanted effects is to restrict GR signaling through defined pathways.

This study has focused on GR interference with two signaling transduction pathways, NF- $\kappa$ B and AP-1, which are involved in a multitude of processes. Given the importance of the GR DBD (see above) with regard to GR cross-talk with these two pathways, we have used GR DBD mutants aiming at separating the GR cross-talk between NF- $\kappa$ B and AP-1. The results obtained demonstrate that it is possible to separate GR cross-talk with these two pathways, and thus restrict GR signaling through defined pathways. Interestingly, GR-mediated upregulation of genes was shown to be involved in glucocorticoid repression of NF- $\kappa$ B activity. In the first study, glucocorticoid upregulation of MKP-1 was demonstrated. GR mediated induction of this gene has previously been suggested to involve putative GREs in the MKP-1 promoter. The presence of functional GREs would be in line with our results obtained with the transactivation deficient GR<sub>R488Q</sub> mutant, but would not explain the results obtained with GR<sub>LS7</sub>. Accordingly, it is important to characterize the promoter and perform functional analysis of putative GREs. This kind of study would benefit from a 'promoter deletion' analysis, like the one performed in paper IV. Moreover, the effects of ERK activation need to be further scrutinized. Using the GR<sub>R488Q</sub> mutant, we could identify a correlation between inactivation of ERK and a functional glucocorticoid repression of NF- $\kappa$ B. However, the downstream substrates/effects of ERK should be examined in more detail. One example of a downstream target of ERK is the nuclear kinase MSK1, which is known to enhance NF- $\kappa$ B activity via phosphorylation of p65 (S276). In addition, the ERK has been suggested to inhibit the activity of GR, which

could fit together with our results. Here, the glucocorticoid-mediated upregulation of MKP-1 would prevent inactivation of the receptor. Accordingly, the GR<sub>R488Q</sub> mutant that is unable to induce MKP-1 expression would be inhibited by the activated ERK.

Based on results obtained using the GRdim mice, the general view is that glucocorticoid repression of genes is sufficient for an anti-inflammatory effect. However, additional studies indicate that the GRdim mutant is capable of activating gene transcription. Experiments performed in vitro demonstrate that the GRdim mutant upregulates the GR dependent phenylethanolamine *N*-methyltransferase (PNMT) gene (Adams et al., 2003). Moreover, findings made in the GRdim mice, which display an unaltered PNMT expression, while GR knockout mice lack PNMT expression, further support for the in vitro observation is provided by the in vivo animal models (Reichardt et al., 1998). Together with our results demonstrating increased gene expression by GR mutants that are unable to transactivate via classical GREs, this indicate that activation of gene expression still can occur via alternative mechanisms. Thus, in addition to glucocorticoid-mediated repression of gene expression, activation of genes may contribute to the antiinflammatory effect (Paper III in this thesis).

The findings made in paper III, highlighted the importance of glucocorticoid inhibition of NF- $\kappa$ B in the antiproliferative response to glucocorticoids. Interestingly, a GR mutant unable to activate transcription through GREs, still activated the I $\kappa$ B $\alpha$  expression, an effect demonstrated to be direct. Together with the fact that no GRE has been identified in the I $\kappa$ B $\alpha$  promoter, this suggests that the GR targets the promoter via binding to an already DNA-bound factor, so called tethering. Performing chromatin immunoprecipitation experiments of different regions of the promoter using a GR antibody could test this hypothesis.

Clearly, more studies are required to define GR cross-talk with NF- $\kappa$ B and AP-1 signaling pathways. Our approach, using GR mutants is one way to address this subject, while screening with different ligands would be an additional approach. Moreover, introducing a GR mutant, which discriminates between NF- $\kappa$ B and AP-1, into an in vivo animal model would obviously give relevant information with regard to the mutant's ability to inhibit biological processes such as inflammation and cell proliferation.

## ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to everyone who has made this thesis possible and especially to:

Professor Jan-Åke Gustafsson for providing me with excellent research facilities at the Department of Medical Nutrition.

Professor Sam Okret, my supervisor, for interesting scientific discussions, for your support and patience and for appreciating my work.

Stefan Nilsson, my co-supervisor, for scientific discussions, for your support and encouragement during times of disbelief.

Rune Toftgård, my co-supervisor, for scientific discussions, especially during the first years.

Professor Keith Yamamoto, for inviting me to his lab and for providing with an inspiring lab environment.

All members of the Keith Yamamoto group, in particular, Hans Luecke, for teaching me the chromatin immunoprecipitation technique, showing me San Francisco and for teaching me the basic rules of baseball

Agneta Mode, for your excellent leadership of the FFB program and for always being positive.

All present and past members of the Sam Okret group, Johan, Magnus, Katarina, Behrouz, Ahmad, Takashi, Gary, Javier and Jacquie. A special thanks goes to Ingalill Rafter, for teaching me different techniques, her patience, and for always being cheerful.

All present and past colleagues at medical nutrition for making the environment at department such a nice place, especially; Tobias, Björn, Katta, Malin, Hanne, Tove, Cissi, Lukas, Lena K, Lena O, Ingrid, Runa, Lotta, Marika, Johanna, Erik and Waffa.

All my friends outside the lab, in particular, my brothers in arms, Ulf, Urban and Marcus, for all the laughter we have shared, for our notorious 24h-breakfasts and for giving me the opportunity to experience Europe, Asia and Africa by motorcycle.

Lunchgänget at Karo Bio, Bosse, Johnny and Johan for giving me the opportunity to discuss important topics in life, such as sport, sport and sport... and for being good friends.

Lars, Gunnel, Monica, Lena, MarieF, MarieJ, Richard, Kerstin, Lady and Ylva, for always being helpful and friendly.

The people at Karo Bio for the great parties and the great time we have shared.

My family, for your constant support and helpfulness throughout the years.

Ulrika, without your love, patience and support this would not have been possible.

Evelina, my daughter, for bringing so much happiness into my life.

## REFERENCES

- Adams, M., Meijer, O.C., Wang, J., Bhargava, A. and Pearce, D. (2003) Homodimerization of the glucocorticoid receptor is not essential for response element binding: activation of the phenylethanolamine N-methyltransferase gene by dimerization-defective mutants. *Mol Endocrinol*, **17**, 2583-92. Epub 2003 Aug 21.
- Adcock, I.M. and Lane, S.J. (2003) Corticosteroid-insensitive asthma: molecular mechanisms. *J Endocrinol*, **178**, 347-55.
- Algarte, M., Kwon, H., Genin, P. and Hiscott, J. (1999) Identification by in vivo genomic footprinting of a transcriptional switch containing NF-kappaB and Sp1 that regulates the IkappaBalpha promoter. *Mol Cell Biol*, **19**, 6140-53.
- Alheim, K., Corness, J., Samuelsson, M.K., Bladh, L.G., Murata, T., Nilsson, T. and Okret, S. (2003) Identification of a functional glucocorticoid response element in the promoter of the cyclin-dependent kinase inhibitor p57Kip2. *J Mol Endocrinol*, **30**, 359-68.
- Almlöf, T., Wright, A.P. and Gustafsson, J.Å. (1995) Role of acidic and phosphorylated residues in gene activation by the glucocorticoid receptor. *J Biol Chem*, **270**, 17535-40.
- Andrews, R.C. and Walker, B.R. (1999) Glucocorticoids and insulin resistance: old hormones, new targets. *Clin Sci (Lond)*, **96**, 513-23.
- Angel, P. and Karin, M. (1991) The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. *Biochim Biophys Acta*, **1072**, 129-57.
- Anzick, S.L., Kononen, J., Walker, R.L., Azorsa, D.O., Tanner, M.M., Guan, X.Y., Sauter, G., Kallioniemi, O.P., Trent, J.M. and Meltzer, P.S. (1997) AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. *Science*, **277**, 965-8.
- Aranda, A. and Pascual, A. (2001) Nuclear hormone receptors and gene expression. *Physiol Rev*, **81**, 1269-304.
- Arias, J., Alberts, A.S., Brindle, P., Claret, F.X., Smeal, T., Karin, M., Feramisco, J. and Montminy, M. (1994) Activation of cAMP and mitogen responsive genes relies on a common nuclear factor. *Nature*, **370**, 226-9.
- Auphan, N., DiDonato, J.A., Rosette, C., Helmborg, A. and Karin, M. (1995) Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis. *Science*, **270**, 286-90.
- Baldwin, A.S., Jr., Azizkhan, J.C., Jensen, D.E., Beg, A.A. and Coodly, L.R. (1991) Induction of NF-kappa B DNA-binding activity during the G0-to-G1 transition in mouse fibroblasts. *Mol Cell Biol*, **11**, 4943-51.
- Bannister, A.J., Oehler, T., Wilhelm, D., Angel, P. and Kouzarides, T. (1995) Stimulation of c-Jun activity by CBP: c-Jun residues Ser63/73 are required for CBP induced stimulation in vivo and CBP binding in vitro. *Oncogene*, **11**, 2509-14.
- Beato, M., Herrlich, P. and Schütz, G. (1995) Steroid hormone receptors: many actors in search of a plot. *Cell*, **83**, 851-7.
- Belvisi, M.G., Wicks, S.L., Battram, C.H., Bottoms, S.E., Redford, J.E., Woodman, P., Brown, T.J., Webber, S.E. and Foster, M.L. (2001) Therapeutic benefit of a

- dissociated glucocorticoid and the relevance of in vitro separation of transrepression from transactivation activity. *J Immunol*, **166**, 1975-82.
- Benoit, G., Malewicz, M. and Perlmann, T. (2004) Digging deep into the pockets of orphan nuclear receptors: insights from structural studies. *Trends Cell Biol*, **14**, 369-76.
- Bladh, L.G., Lidén, J., Pazirandeh, A., Rafter, I., Dahlman-Wright, K., Nilsson, S. and Okret, S. (2005) Identification of Target Genes Involved in the Antiproliferative Effect of Glucocorticoids Reveals a Role for Nuclear Factor- $\{\kappa\}$ B Repression. *Mol Endocrinol*, **19**, 632-43 Epub 2004 Nov 04.
- Bledsoe, R.K., Montana, V.G., Stanley, T.B., Delves, C.J., Apolito, C.J., McKee, D.D., Consler, T.G., Parks, D.J., Stewart, E.L., Willson, T.M., Lambert, M.H., Moore, J.T., Pearce, K.H. and Xu, H.E. (2002) Crystal structure of the glucocorticoid receptor ligand binding domain reveals a novel mode of receptor dimerization and coactivator recognition. *Cell*, **110**, 93-105.
- Boruk, M., Savory, J.G. and Haché, R.J. (1998) AF-2-dependent potentiation of CCAAT enhancer binding protein beta-mediated transcriptional activation by glucocorticoid receptor. *Mol Endocrinol*, **12**, 1749-63.
- Brostjan, C., Anrather, J., Csizmadia, V., Stroka, D., Soares, M., Bach, F.H. and Winkler, H. (1996) Glucocorticoid-mediated repression of NF $\kappa$ B activity in endothelial cells does not involve induction of IkappaBalpha synthesis. *J Biol Chem*, **271**, 19612-6.
- Brown, K., Gerstberger, S., Carlson, L., Franzoso, G. and Siebenlist, U. (1995) Control of I kappa B-alpha proteolysis by site-specific, signal-induced phosphorylation. *Science*, **267**, 1485-8.
- Bruna, A., Nicolas, M., Munoz, A., Kyriakis, J.M. and Caelles, C. (2003) Glucocorticoid receptor-JNK interaction mediates inhibition of the JNK pathway by glucocorticoids. *Embo J*, **22**, 6035-44.
- Caelles, C., Gonzalez-Sancho, J.M. and Munoz, A. (1997) Nuclear hormone receptor antagonism with AP-1 by inhibition of the JNK pathway. *Genes Dev*, **11**, 3351-64.
- Caldenhoven, E., Lidén, J., Wissink, S., Van de Stolpe, A., Raaijmakers, J., Koenderman, L., Okret, S., Gustafsson, J.Å. and 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.
- Carlstedt-Duke, J. (1999) Glucocorticoid Receptor beta: View II. *Trends Endocrinol Metab*, **10**, 339-342.
- Carlstedt-Duke, J., Okret, S., Wrangé, Ö. and Gustafsson, J.Å. (1982) Immunochemical analysis of the glucocorticoid receptor: identification of a third domain separate from the steroid-binding and DNA-binding domains. *Proc Natl Acad Sci U S A*, **79**, 4260-4.
- Chapman, M.S., Askew, D.J., Kuscuoglu, U. and Miesfeld, R.L. (1996) Transcriptional control of steroid-regulated apoptosis in murine thymoma cells. *Mol Endocrinol*, **10**, 967-78.
- Chawla, A., Repa, J.J., Evans, R.M. and Mangelsdorf, D.J. (2001) Nuclear receptors and lipid physiology: opening the X-files. *Science*, **294**, 1866-70.
- Chen, F., Castranova, V. and Shi, X. (2001) New insights into the role of nuclear factor-kappaB in cell growth regulation. *Am J Pathol*, **159**, 387-97.

- Chen, J.D. and Evans, R.M. (1995) A transcriptional co-repressor that interacts with nuclear hormone receptors. *Nature*, **377**, 454-7.
- Cole, T.J., Blendy, J.A., Monaghan, A.P., Krieglstein, K., Schmid, W., Aguzzi, A., Fantuzzi, G., Hummler, E., Unsicker, K. and Schütz, G. (1995) Targeted disruption of the glucocorticoid receptor gene blocks adrenergic chromaffin cell development and severely retards lung maturation. *Genes Dev*, **9**, 1608-21.
- Committee, N.R. (1999) A unified nomenclature system for the nuclear receptor superfamily. *Cell*, **97**, 161-3.
- Corroyer, S., Nabeyrat, E. and Clement, A. (1997) Involvement of the cell cycle inhibitor CIP1/WAF1 in lung alveolar epithelial cell growth arrest induced by glucocorticoids. *Endocrinology*, **138**, 3677-85.
- Dahlman-Wright, K., Wright, A., Gustafsson, J.Å. and Carlstedt-Duke, J. (1991) Interaction of the glucocorticoid receptor DNA-binding domain with DNA as a dimer is mediated by a short segment of five amino acids. *J Biol Chem*, **266**, 3107-12.
- Danielsen, M., Northrop, J.P. and Ringold, G.M. (1986) The mouse glucocorticoid receptor: mapping of functional domains by cloning, sequencing and expression of wild-type and mutant receptor proteins. *Embo J*, **5**, 2513-22.
- Davani, B., Khan, A., Hult, M., Mårtensson, E., Okret, S., Efendic, S., Jörnvall, H. and Oppermann, U.C. (2000) Type 1 11beta -hydroxysteroid dehydrogenase mediates glucocorticoid activation and insulin release in pancreatic islets. *J Biol Chem*, **275**, 34841-4.
- Davies, T.H., Ning, Y.M. and Sanchez, E.R. (2002) A new first step in activation of steroid receptors: hormone-induced switching of FKBP51 and FKBP52 immunophilins. *J Biol Chem*, **277**, 4597-600. Epub 2001 Dec 20.
- De Bosscher, K., Vanden Berghe, W. and Haegeman, G. (2001) Glucocorticoid repression of AP-1 is not mediated by competition for nuclear coactivators. *Mol Endocrinol*, **15**, 219-27.
- De Bosscher, K., Vanden Berghe, W. and Haegeman, G. (2003) The interplay between the glucocorticoid receptor and nuclear factor-kappaB or activator protein-1: molecular mechanisms for gene repression. *Endocr Rev*, **24**, 488-522.
- De Bosscher, K., Vanden Berghe, W., Vermeulen, L., Plaisance, S., Boone, E. and Haegeman, G. (2000) Glucocorticoids repress NF-kappaB-driven genes by disturbing the interaction of p65 with the basal transcription machinery, irrespective of coactivator levels in the cell. *Proc Natl Acad Sci U S A*, **97**, 3919-24.
- Deak, M., Clifton, A.D., Lucocq, L.M. and Alessi, D.R. (1998) Mitogen- and stress-activated protein kinase-1 (MSK1) is directly activated by MAPK and SAPK2/p38, and may mediate activation of CREB. *Embo J*, **17**, 4426-41.
- Delerive, P., De Bosscher, K., Vanden Berghe, W., Fruchart, J.C., Haegeman, G. and Staels, B. (2002) DNA binding-independent induction of IkappaBalpha gene transcription by PPARalpha. *Mol Endocrinol*, **16**, 1029-39.
- Delerive, P., Gervois, P., Fruchart, J.C. and Staels, B. (2000) Induction of IkappaBalpha expression as a mechanism contributing to the anti-inflammatory activities of peroxisome proliferator-activated receptor-alpha activators. *J Biol Chem*, **275**, 36703-7.
- Diamond, M.I., Miner, J.N., Yoshinaga, S.K. and Yamamoto, K.R. (1990) Transcription factor interactions: selectors of positive or negative regulation from a single DNA element. *Science*, **249**, 1266-72.

- Dong, Y., Poellinger, L., Gustafsson, J.Å. and Okret, S. (1988) Regulation of glucocorticoid receptor expression: evidence for transcriptional and posttranslational mechanisms. *Mol Endocrinol*, **2**, 1256-64.
- Doucas, V., Shi, Y., Miyamoto, S., West, A., Verma, I. and 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 U S A*, **97**, 11893-8.
- Drouin, J., Trifiro, M.A., Plante, R.K., Nemer, M., Eriksson, P. and Wrange, Ö. (1989) Glucocorticoid receptor binding to a specific DNA sequence is required for hormone-dependent repression of pro-opiomelanocortin gene transcription. *Mol Cell Biol*, **9**, 5305-14.
- Elbi, C., Walker, D.A., Romero, G., Sullivan, W.P., Toft, D.O., Hager, G.L. and DeFranco, D.B. (2004) Molecular chaperones function as steroid receptor nuclear mobility factors. *Proc Natl Acad Sci U S A*, **101**, 2876-81 Epub 2004 Feb 20.
- Engelbrecht, Y., de Wet, H., Horsch, K., Langeveldt, C.R., Hough, F.S. and Hulley, P.A. (2003) Glucocorticoids induce rapid up-regulation of mitogen-activated protein kinase phosphatase-1 and dephosphorylation of extracellular signal-regulated kinase and impair proliferation in human and mouse osteoblast cell lines. *Endocrinology*, **144**, 412-22.
- Findling, J.W., Aron, D.C. and Tyrell, J.B. (1997) Glucocorticoids and Adrenal Androgens. In: Greenspan, F.S. and Strewler, G.J. (Eds), *Basic and Clinical Endocrinology*. Appleton & Lange, Stamford, 317-358.
- Firestein, G.S. and Manning, A.M. (1999) Signal transduction and transcription factors in rheumatic disease. *Arthritis Rheum*, **42**, 609-21.
- Fischle, W., Wang, Y. and Allis, C.D. (2003) Histone and chromatin cross-talk. *Curr Opin Cell Biol*, **15**, 172-83.
- Ford, J., McEwan, I.J., Wright, A.P. and Gustafsson, J.Å. (1997) Involvement of the transcription factor IID protein complex in gene activation by the N-terminal transactivation domain of the glucocorticoid receptor in vitro. *Mol Endocrinol*, **11**, 1467-75.
- Freeman, B.C. and Yamamoto, K.R. (2002) Disassembly of transcriptional regulatory complexes by molecular chaperones. *Science*, **296**, 2232-5.
- Galigniana, M.D., Scruggs, J.L., Herrington, J., Welsh, M.J., Carter-Su, C., Housley, P.R. and Pratt, W.B. (1998) Heat shock protein 90-dependent (geldanamycin-inhibited) movement of the glucocorticoid receptor through the cytoplasm to the nucleus requires intact cytoskeleton. *Mol Endocrinol*, **12**, 1903-13.
- Garrington, T.P. and Johnson, G.L. (1999) Organization and regulation of mitogen-activated protein kinase signaling pathways. *Curr Opin Cell Biol*, **11**, 211-8.
- Gerritsen, M.E., Williams, A.J., Neish, A.S., Moore, S., Shi, Y. and Collins, T. (1997) CREB-binding protein/p300 are transcriptional coactivators of p65. *Proc Natl Acad Sci U S A*, **94**, 2927-32.
- Ghosh, S. and Karin, M. (2002) Missing pieces in the NF-kappaB puzzle. *Cell*, **109**, S81-96.
- Grab, L.T., Kearns, M.W., Morris, A.J. and Daniel, L.W. (2004) Differential role for phospholipase D1 and phospholipase D2 in 12-O-tetradecanoyl-13-phorbol acetate-stimulated MAPK activation, Cox-2 and IL-8 expression. *Biochim Biophys Acta*, **1636**, 29-39.

- Gronemeyer, H., Gustafsson, J.Å. and Laudet, V. (2004) Principles for modulation of the nuclear receptor superfamily. *Nat Rev Drug Discov*, **3**, 950-64.
- Guttridge, D.C., Albanese, C., Reuther, J.Y., Pestell, R.G. and Baldwin, A.S., Jr. (1999) NF-kappaB controls cell growth and differentiation through transcriptional regulation of cyclin D1. *Mol Cell Biol*, **19**, 5785-99.
- Hayden, M.S. and Ghosh, S. (2004) Signaling to NF-kappaB. *Genes Dev*, **18**, 2195-224.
- Hebbar, P.B. and Archer, T.K. (2003) Chromatin remodeling by nuclear receptors. *Chromosoma*, **111**, 495-504 Epub 2003 Feb 26.
- Heck, S., Bender, K., Kullmann, M., Göttlicher, M., Herrlich, P. and 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. and 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**, 4087-95.
- Heery, D.M., Kalkhoven, E., Hoare, S. and Parker, M.G. (1997) A signature motif in transcriptional co-activators mediates binding to nuclear receptors. *Nature*, **387**, 733-6.
- Helmberg, A., Auphan, N., Caelles, C. and 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.
- Herzig, S., Long, F., Jhala, U.S., Hedrick, S., Quinn, R., Bauer, A., Rudolph, D., Schütz, G., Yoon, C., Puigserver, P., Spiegelman, B. and Montminy, M. (2001) CREB regulates hepatic gluconeogenesis through the coactivator PGC-1. *Nature*, **413**, 179-83.
- Hinz, M., Krappmann, D., Eichten, A., Heder, A., Scheidereit, C. and Strauss, M. (1999) NF-kappaB function in growth control: regulation of cyclin D1 expression and G0/G1-to-S-phase transition. *Mol Cell Biol*, **19**, 2690-8.
- Hollenberg, S.M., Weinberger, C., Ong, E.S., Cerelli, G., Oro, A., Lebo, R., Thompson, E.B., Rosenfeld, M.G. and Evans, R.M. (1985) Primary structure and expression of a functional human glucocorticoid receptor cDNA. *Nature*, **318**, 635-41.
- Imai, E., Strömstedt, P.E., Quinn, P.G., Carlstedt-Duke, J., Gustafsson, J.Å. and Granner, D.K. (1990) Characterization of a complex glucocorticoid response unit in the phosphoenolpyruvate carboxykinase gene. *Mol Cell Biol*, **10**, 4712-9.
- Ismaili, N. and Garabedian, M.J. (2004) Modulation of glucocorticoid receptor function via phosphorylation. *Ann N Y Acad Sci*, **1024**, 86-101.
- Ito, K., Barnes, P.J. and Adcock, I.M. (2000) Glucocorticoid receptor recruitment of histone deacetylase 2 inhibits interleukin-1beta-induced histone H4 acetylation on lysines 8 and 12. *Mol Cell Biol*, **20**, 6891-903.
- Jenkins, B.D., Pullen, C.B. and Darimont, B.D. (2001) Novel glucocorticoid receptor coactivator effector mechanisms. *Trends Endocrinol Metab*, **12**, 122-6.
- Jonat, C., Rahmsdorf, H.J., Park, K.K., Cato, A.C., Gebel, S., Ponta, H. and Herrlich, P. (1990) Antitumor promotion and antiinflammation: down-modulation of AP-1 (Fos/Jun) activity by glucocorticoid hormone. *Cell*, **62**, 1189-204.



- Kaltschmidt, B., Kaltschmidt, C., Hehner, S.P., Droge, W. and Schmitz, M.L. (1999) Repression of NF-kappaB impairs HeLa cell proliferation by functional interference with cell cycle checkpoint regulators. *Oncogene*, **18**, 3213-25.
- Kamei, Y., Xu, L., Heinzl, T., Torchia, J., Kurokawa, R., Gloss, B., Lin, S.C., Heyman, R.A., Rose, D.W., Glass, C.K. and Rosenfeld, M.G. (1996) A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. *Cell*, **85**, 403-14.
- Karin, M. (2004) Mitogen activated protein kinases as targets for development of novel anti-inflammatory drugs. *Ann Rheum Dis*, **63**, ii62-ii64.
- Karin, M. and Ben-Neriah, Y. (2000) Phosphorylation meets ubiquitination: the control of NF-[kappa]B activity. *Annu Rev Immunol*, **18**, 621-63.
- Karin, M., Cao, Y., Greten, F.R. and Li, Z.W. (2002) NF-kappaB in cancer: from innocent bystander to major culprit. *Nat Rev Cancer*, **2**, 301-10.
- Karin, M., Liu, Z. and Zandi, E. (1997) AP-1 function and regulation. *Curr Opin Cell Biol*, **9**, 240-6.
- Kassel, O., Sancono, A., Kratzschmar, J., Kreft, B., Stassen, M. and Cato, A.C. (2001) Glucocorticoids inhibit MAP kinase via increased expression and decreased degradation of MKP-1. *Embo J*, **20**, 7108-16.
- Kauppi, B., Jakob, C., Färnegårdh, M., Yang, J., Ahola, H., Alarcon, M., Calles, K., Engström, O., Harlan, J., Muchmore, S., Ramqvist, A.K., Thorell, S., Öhman, L., Greer, J., Gustafsson, J.Å., Carlstedt-Duke, J. and Carlquist, M. (2003) The three-dimensional structures of antagonistic and agonistic forms of the glucocorticoid receptor ligand-binding domain: RU-486 induces a transconformation that leads to active antagonism. *J Biol Chem*, **278**, 22748-54 Epub 2003 Apr 09.
- Kinyamu, H.K. and Archer, T.K. (2004) Modifying chromatin to permit steroid hormone receptor-dependent transcription. *Biochim Biophys Acta*, **1677**, 30-45.
- König, H., Ponta, H., Rahmsdorf, H.J. and Herrlich, P. (1992) Interference between pathway-specific transcription factors: glucocorticoids antagonize phorbol ester-induced AP-1 activity without altering AP-1 site occupation in vivo. *Embo J*, **11**, 2241-6.
- Lasa, M., Brook, M., Saklatvala, J. and Clark, A.R. (2001) Dexamethasone destabilizes cyclooxygenase 2 mRNA by inhibiting mitogen-activated protein kinase p38. *Mol Cell Biol*, **21**, 771-80.
- Laudet, V. (1997) Evolution of the nuclear receptor superfamily: early diversification from an ancestral orphan receptor. *J Mol Endocrinol*, **19**, 207-26.
- Lee, S.K., Kim, H.J., Na, S.Y., Kim, T.S., Choi, H.S., Im, S.Y. and Lee, J.W. (1998) Steroid receptor coactivator-1 coactivates activating protein-1-mediated transactivations through interaction with the c-Jun and c-Fos subunits. *J Biol Chem*, **273**, 16651-4.
- Li, L.B., Goleva, E., Hall, C.F., Ou, L.S. and Leung, D.Y. (2004) Superantigen-induced corticosteroid resistance of human T cells occurs through activation of the mitogen-activated protein kinase kinase/extracellular signal-regulated kinase (MEK-ERK) pathway. *J Allergy Clin Immunol*, **114**, 1059-69.
- Lidén, J., Delaunay, F., Rafter, I., Gustafsson, J. and 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.

- Lidén, J., Rafter, I., Truss, M., Gustafsson, J.Å. and Okret, S. (2000) Glucocorticoid effects on NF-kappaB binding in the transcription of the ICAM-1 gene. *Biochem Biophys Res Commun*, **273**, 1008-14.
- Liu, J. and DeFranco, D.B. (1999) Chromatin recycling of glucocorticoid receptors: implications for multiple roles of heat shock protein 90. *Mol Endocrinol*, **13**, 355-65.
- Liu, W., Wang, J., Sauter, N.K. and Pearce, D. (1995) Steroid receptor heterodimerization demonstrated in vitro and in vivo. *Proc Natl Acad Sci U S A*, **92**, 12480-4.
- Luger, K., Mader, A.W., Richmond, R.K., Sargent, D.F. and Richmond, T.J. (1997) Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature*, **389**, 251-60.
- Luisi, B.F., Xu, W.X., Otwinowski, Z., Freedman, L.P., Yamamoto, K.R. and Sigler, P.B. (1991) Crystallographic analysis of the interaction of the glucocorticoid receptor with DNA. *Nature*, **352**, 497-505.
- Malik, S. and Roeder, R.G. (2000) Transcriptional regulation through Mediator-like coactivators in yeast and metazoan cells. *Trends Biochem Sci*, **25**, 277-83.
- Mangelsdorf, D.J., Thummel, C., Beato, M., Herrlich, P., Schütz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M., Chambon, P. and et al. (1995) The nuclear receptor superfamily: the second decade. *Cell*, **83**, 835-9.
- Masuzaki, H., Paterson, J., Shinyama, H., Morton, N.M., Mullins, J.J., Seckl, J.R. and Flier, J.S. (2001) A transgenic model of visceral obesity and the metabolic syndrome. *Science*, **294**, 2166-70.
- McKay, L.I. and Cidlowski, J.A. (2000) CBP (CREB binding protein) integrates NF-kappaB (nuclear factor-kappaB) and glucocorticoid receptor physical interactions and antagonism. *Mol Endocrinol*, **14**, 1222-34.
- McNally, J.G., Muller, W.G., Walker, D., Wolford, R. and Hager, G.L. (2000) The glucocorticoid receptor: rapid exchange with regulatory sites in living cells. *Science*, **287**, 1262-5.
- Mechta-Grigoriou, F., Gerald, D. and Yaniv, M. (2001) The mammalian Jun proteins: redundancy and specificity. *Oncogene*, **20**, 2378-89.
- Miesfeld, R., Okret, S., Wikström, A.C., Wrangé, Ö., Gustafsson, J.Å. and Yamamoto, K.R. (1984) Characterization of a steroid hormone receptor gene and mRNA in wild-type and mutant cells. *Nature*, **312**, 779-81.
- Miesfeld, R., Rusconi, S., Godowski, P.J., Maler, B.A., Okret, S., Wikström, A.C., Gustafsson, J.Å. and Yamamoto, K.R. (1986) Genetic complementation of a glucocorticoid receptor deficiency by expression of cloned receptor cDNA. *Cell*, **46**, 389-99.
- Na, S.Y., Lee, S.K., Han, S.J., Choi, H.S., Im, S.Y. and Lee, J.W. (1998) Steroid receptor coactivator-1 interacts with the p50 subunit and coactivates nuclear factor kappaB-mediated transactivations. *J Biol Chem*, **273**, 10831-4.
- Narlikar, G.J., Fan, H.Y. and Kingston, R.E. (2002) Cooperation between complexes that regulate chromatin structure and transcription. *Cell*, **108**, 475-87.
- Nissen, R.M. and 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**, 2314-29.
- Ohtsuka, T., Kubota, A., Hirano, T., Watanabe, K., Yoshida, H., Tsurufuji, M., Iizuka, Y., Konishi, K. and Tsurufuji, S. (1996) Glucocorticoid-mediated gene suppression of rat cytokine-induced neutrophil chemoattractant CINC/gro, a

- member of the interleukin-8 family, through impairment of NF-kappa B activation. *J Biol Chem*, **271**, 1651-9.
- Okret, S., Dong, Y., Brönnegard, M. and Gustafsson, J.Å. (1991) Regulation of glucocorticoid receptor expression. *Biochimie*, **73**, 51-9.
- Onate, S.A., Tsai, S.Y., Tsai, M.J. and O'Malley, B.W. (1995) Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. *Science*, **270**, 1354-7.
- Otsuka, G., Nagaya, T., Saito, K., Mizuno, M., Yoshida, J. and Seo, H. (1999) Inhibition of nuclear factor-kappaB activation confers sensitivity to tumor necrosis factor-alpha by impairment of cell cycle progression in human glioma cells. *Cancer Res*, **59**, 4446-52.
- Pascual, L.E.T.L., Simone, F., Viengchareun, S., Meduri, G., Thirman, M.J. and Lombes, M. (2005) The elongation factor ELL is a selective coregulator for steroid receptor functions. *Mol Endocrinol*, **14**, 14.
- Pearce, D. and Yamamoto, K.R. (1993) Mineralocorticoid and glucocorticoid receptor activities distinguished by nonreceptor factors at a composite response element. *Science*, **259**, 1161-5.
- Pearson, G., Robinson, F., Beers Gibson, T., Xu, B.E., Karandikar, M., Berman, K. and Cobb, M.H. (2001) Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. *Endocr Rev*, **22**, 153-83.
- Perissi, V., Staszewski, L.M., McInerney, E.M., Kurokawa, R., Krones, A., Rose, D.W., Lambert, M.H., Milburn, M.V., Glass, C.K. and Rosenfeld, M.G. (1999) Molecular determinants of nuclear receptor-corepressor interaction. *Genes Dev*, **13**, 3198-208.
- Pratt, W.B., Galigniana, M.D., Harrell, J.M. and DeFranco, D.B. (2004) Role of hsp90 and the hsp90-binding immunophilins in signalling protein movement. *Cell Signal*, **16**, 857-72.
- Pratt, W.B. and Toft, D.O. (1997) Steroid receptor interactions with heat shock protein and immunophilin chaperones. *Endocr Rev*, **18**, 306-60.
- Pugeat, M., Bonneton, A., Perrot, D., Rocle-Nicolas, B., Lejeune, H., Grenot, C., Dechaud, H., Brebant, C., Motin, J. and Cuilleron, C.Y. (1989) Decreased immunoreactivity and binding activity of corticosteroid-binding globulin in serum in septic shock. *Clin Chem*, **35**, 1675-9.
- Rachez, C., Lemon, B.D., Suldan, Z., Bromleigh, V., Gamble, M., Naar, A.M., Erdjument-Bromage, H., Tempst, P. and Freedman, L.P. (1999) Ligand-dependent transcription activation by nuclear receptors requires the DRIP complex. *Nature*, **398**, 824-8.
- Ray, A. and Prefontaine, K.E. (1994) Physical association and functional antagonism between the p65 subunit of transcription factor NF-kappa B and the glucocorticoid receptor. *Proc Natl Acad Sci U S A*, **91**, 752-6.
- Reichardt, H.M., Kaestner, K.H., Tuckermann, J., Kretz, O., Wessely, O., Bock, R., Gass, P., Schmid, W., Herrlich, P., Angel, P. and Schütz, G. (1998) DNA binding of the glucocorticoid receptor is not essential for survival. *Cell*, **93**, 531-41.
- Reichardt, H.M. and Schütz, G. (1998) Glucocorticoid signalling--multiple variations of a common theme. *Mol Cell Endocrinol*, **146**, 1-6.
- Reichardt, H.M., Tuckermann, J.P., Göttlicher, M., Vujic, M., Weih, F., Angel, P., Herrlich, P. and Schütz, G. (2001) Repression of inflammatory responses in the absence of DNA binding by the glucocorticoid receptor. *Embo J*, **20**, 7168-73.

- Rider, L.G., Hirasawa, N., Santini, F. and Beaven, M.A. (1996) Activation of the mitogen-activated protein kinase cascade is suppressed by low concentrations of dexamethasone in mast cells. *J Immunol*, **157**, 2374-80.
- Rogatsky, I., Logan, S.K. and Garabedian, M.J. (1998) Antagonism of glucocorticoid receptor transcriptional activation by the c-Jun N-terminal kinase. *Proc Natl Acad Sci U S A*, **95**, 2050-5.
- Rogatsky, I., Trowbridge, J.M. and Garabedian, M.J. (1997) Glucocorticoid receptor-mediated cell cycle arrest is achieved through distinct cell-specific transcriptional regulatory mechanisms. *Mol Cell Biol*, **17**, 3181-93.
- Rogatsky, I., Zarembek, K.A. and Yamamoto, K.R. (2001) Factor recruitment and TIF2/GRIP1 corepressor activity at a collagenase-3 response element that mediates regulation by phorbol esters and hormones. *Embo J*, **20**, 6071-83.
- Ruiz, M., Lind, U., Gåfvels, M., Eggertsen, G., Carlstedt-Duke, J., Nilsson, L., Holtmann, M., Stiern, P., Wikström, A.C. and Werner, S. (2001) Characterization of two novel mutations in the glucocorticoid receptor gene in patients with primary cortisol resistance. *Clin Endocrinol (Oxf)*, **55**, 363-71.
- Sadikot, R.T., Christman, J.W. and Blackwell, T.S. (2004) Molecular targets for modulating lung inflammation and injury. *Curr Drug Targets*, **5**, 581-8.
- Sakurai, H., Chiba, H., Miyoshi, H., Sugita, T. and Toriumi, W. (1999) I $\kappa$ B kinases phosphorylate NF- $\kappa$ B p65 subunit on serine 536 in the transactivation domain. *J Biol Chem*, **274**, 30353-6.
- Samuelsson, M.K., Pazirandeh, A., Davani, B. and Okret, S. (1999) p57Kip2, a glucocorticoid-induced inhibitor of cell cycle progression in HeLa cells. *Mol Endocrinol*, **13**, 1811-22.
- Sanchez, I., Goya, L., Vallerga, A.K. and Firestone, G.L. (1993) Glucocorticoids reversibly arrest rat hepatoma cell growth by inducing an early G1 block in cell cycle progression. *Cell Growth Differ*, **4**, 215-25.
- Scheinman, R.I., Cogswell, P.C., Lofquist, A.K. and Baldwin, A.S., Jr. (1995) Role of transcriptional activation of I $\kappa$ B $\alpha$  in mediation of immunosuppression by glucocorticoids. *Science*, **270**, 283-6.
- Schreiber, M., Kolbus, A., Piu, F., Szabowski, A., Mohle-Steinlein, U., Tian, J., Karin, M., Angel, P. and Wagner, E.F. (1999) Control of cell cycle progression by c-Jun is p53 dependent. *Genes Dev*, **13**, 607-19.
- Schulz, M., Eggert, M., Baniahmad, A., Dostert, A., Heinzl, T. and Renkawitz, R. (2002) RU486-induced glucocorticoid receptor agonism is controlled by the receptor N terminus and by corepressor binding. *J Biol Chem*, **277**, 26238-43 Epub 2002 May 13.
- Schüle, R., Rangarajan, P., Kliewer, S., Ransone, L.J., Bolado, J., Yang, N., Verma, I.M. and Evans, R.M. (1990) Functional antagonism between oncoprotein c-Jun and the glucocorticoid receptor. *Cell*, **62**, 1217-26.
- Schäcke, H., Docke, W.D. and Asadullah, K. (2002) Mechanisms involved in the side effects of glucocorticoids. *Pharmacol Ther*, **96**, 23-43.
- Shang, Y., Hu, X., DiRenzo, J., Lazar, M.A. and Brown, M. (2000) Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription. *Cell*, **103**, 843-52.
- Shaulian, E. and Karin, M. (2001) AP-1 in cell proliferation and survival. *Oncogene*, **20**, 2390-400.
- Shaulian, E. and Karin, M. (2002) AP-1 as a regulator of cell life and death. *Nat Cell Biol*, **4**, E131-6.

- Sheppard, K.A., Phelps, K.M., Williams, A.J., Thanos, D., Glass, C.K., Rosenfeld, M.G., Gerritsen, M.E. and 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.
- Sherr, C.J. and Roberts, J.M. (1999) CDK inhibitors: positive and negative regulators of G1-phase progression. *Genes Dev*, **13**, 1501-12.
- Smith, C.L. and O'Malley, B.W. (2004) Coregulator function: a key to understanding tissue specificity of selective receptor modulators. *Endocr Rev*, **25**, 45-71.
- Smith, E., Redman, R.A., Logg, C.R., Coetzee, G.A., Kasahara, N. and Frenkel, B. (2000) Glucocorticoids inhibit developmental stage-specific osteoblast cell cycle. Dissociation of cyclin A-cyclin-dependent kinase 2 from E2F4-p130 complexes. *J Biol Chem*, **275**, 19992-20001.
- Stafford, J.M., Wilkinson, J.C., Beechem, J.M. and Granner, D.K. (2001) Accessory factors facilitate the binding of glucocorticoid receptor to the phosphoenolpyruvate carboxykinase gene promoter. *J Biol Chem*, **276**, 39885-91 Epub 2001 Aug 22.
- Stein, B. and Yang, M.X. (1995) Repression of the interleukin-6 promoter by estrogen receptor is mediated by NF-kappa B and C/EBP beta. *Mol Cell Biol*, **15**, 4971-9.
- Stevens, A., Garside, H., Berry, A., Waters, C., White, A. and Ray, D. (2003) Dissociation of steroid receptor coactivator 1 and nuclear receptor corepressor recruitment to the human glucocorticoid receptor by modification of the ligand-receptor interface: the role of tyrosine 735. *Mol Endocrinol*, **17**, 845-59 Epub 2003 Jan 30.
- Strömstedt, P.E., Poellinger, L., Gustafsson, J.Å. and Carlstedt-Duke, J. (1991) The glucocorticoid receptor binds to a sequence overlapping the TATA box of the human osteocalcin promoter: a potential mechanism for negative regulation. *Mol Cell Biol*, **11**, 3379-83.
- Stöcklin, E., Wissler, M., Gouilleux, F. and Groner, B. (1996) Functional interactions between Stat5 and the glucocorticoid receptor. *Nature*, **383**, 726-8.
- Subramaniam, N., Cairns, W. and Okret, S. (1997) Studies on the mechanism of glucocorticoid-mediated repression from a negative glucocorticoid response element from the bovine prolactin gene. *DNA Cell Biol*, **16**, 153-63.
- Subramaniam, N., Champion, J., Rafter, I. and Okret, S. (2003) Cross-talk between glucocorticoid and retinoic acid signals involving glucocorticoid receptor interaction with the homoeodomain protein Pbx1. *Biochem J*, **370**, 1087-95.
- Theodosiou, A. and Ashworth, A. (2002) MAP kinase phosphatases. *Genome Biol*, **3**, REVIEWS3009 Epub 2002 Jun 26.
- Thompson, E.B., Nazareth, L.V., Thulasi, R., Ashraf, J., Harbour, D. and Johnson, B.H. (1992) Glucocorticoids in malignant lymphoid cells: gene regulation and the minimum receptor fragment for lysis. *J Steroid Biochem Mol Biol*, **41**, 273-82.
- Trapp, T., Rupprecht, R., Castren, M., Reul, J.M. and Holsboer, F. (1994) Heterodimerization between mineralocorticoid and glucocorticoid receptor: a new principle of glucocorticoid action in the CNS. *Neuron*, **13**, 1457-62.
- Truss, M. and Beato, M. (1993) Steroid hormone receptors: interaction with deoxyribonucleic acid and transcription factors. *Endocr Rev*, **14**, 459-79.

- Tsigos, C., Kyrou, I., Chrousos, G.P. and Papanicolaou, D.A. (1998) Prolonged suppression of corticosteroid-binding globulin by recombinant human interleukin-6 in man. *J Clin Endocrinol Metab*, **83**, 3379.
- Tsitoura, D.C. and Rothman, P.B. (2004) Enhancement of MEK/ERK signaling promotes glucocorticoid resistance in CD4+ T cells. *J Clin Invest*, **113**, 619-27.
- Walker, D., Htun, H. and Hager, G.L. (1999) Using inducible vectors to study intracellular trafficking of GFP-tagged steroid/nuclear receptors in living cells. *Methods*, **19**, 386-93.
- Wang, T.C., Cardiff, R.D., Zukerberg, L., Lees, E., Arnold, A. and Schmidt, E.V. (1994) Mammary hyperplasia and carcinoma in MMTV-cyclin D1 transgenic mice. *Nature*, **369**, 669-71.
- Wang, Z., Benoit, G., Liu, J., Prasad, S., Aarnisalo, P., Liu, X., Xu, H., Walker, N.P. and Perlmann, T. (2003) Structure and function of Nurr1 identifies a class of ligand-independent nuclear receptors. *Nature*, **423**, 555-60.
- Wang, Z., Frederick, J. and Garabedian, M.J. (2002) Deciphering the phosphorylation "code" of the glucocorticoid receptor in vivo. *J Biol Chem*, **277**, 26573-80 Epub 2002 May 08.
- Vayssiere, B.M., Dupont, S., Choquart, A., Petit, F., Garcia, T., Marchandeu, C., Gronemeyer, H. and Resche-Rigon, M. (1997) Synthetic glucocorticoids that dissociate transactivation and AP-1 transrepression exhibit antiinflammatory activity in vivo. *Mol Endocrinol*, **11**, 1245-55.
- Webster, J.C., Jewell, C.M., Bodwell, J.E., Munck, A., Sar, M. and Cidlowski, J.A. (1997) Mouse glucocorticoid receptor phosphorylation status influences multiple functions of the receptor protein. *J Biol Chem*, **272**, 9287-93.
- Vermeulen, K., Van Bockstaele, D.R. and Berneman, Z.N. (2003a) The cell cycle: a review of regulation, deregulation and therapeutic targets in cancer. *Cell Prolif*, **36**, 131-49.
- Vermeulen, L., De Wilde, G., Van Damme, P., Vanden Berghe, W. and Haegeman, G. (2003b) Transcriptional activation of the NF-kappaB p65 subunit by mitogen- and stress-activated protein kinase-1 (MSK1). *Embo J*, **22**, 1313-24.
- Wisdom, R., Johnson, R.S. and Moore, C. (1999) c-Jun regulates cell cycle progression and apoptosis by distinct mechanisms. *Embo J*, **18**, 188-97.
- Wissink, S., van Heerde, E.C., Schmitz, M.L., Kalkhoven, E., van der Burg, B., Baeuerle, P.A. and 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.
- Voegel, J.J., Heine, M.J., Zechel, C., Chambon, P. and Gronemeyer, H. (1996) TIF2, a 160 kDa transcriptional mediator for the ligand-dependent activation function AF-2 of nuclear receptors. *Embo J*, **15**, 3667-75.
- Vottero, A. and Chrousos, G.P. (1999) Glucocorticoid Receptor beta: View I. *Trends Endocrinol Metab*, **10**, 333-338.
- Woychik, N.A. and Hampsey, M. (2002) The RNA polymerase II machinery: structure illuminates function. *Cell*, **108**, 453-63.
- Wrangé, Ö. and Gustafsson, J.Å. (1978) Separation of the hormone- and DNA-binding sites of the hepatic glucocorticoid receptor by means of proteolysis. *J Biol Chem*, **253**, 856-65.
- Wu, J., Li, Y., Dietz, J. and Lala, D.S. (2004a) Repression of p65 transcriptional activation by the glucocorticoid receptor in the absence of receptor-coactivator interactions. *Mol Endocrinol*, **18**, 53-62 Epub 2003 Oct 9.

- Wu, J.T. and Kral, J.G. (2005) The NF-kappaB/IkappaB signaling system: A molecular target in breast cancer therapy. *J Surg Res*, **123**, 158-169.
- Wu, W., Chaudhuri, S., Brickley, D.R., Pang, D., Karrison, T. and Conzen, S.D. (2004b) Microarray analysis reveals glucocorticoid-regulated survival genes that are associated with inhibition of apoptosis in breast epithelial cells. *Cancer Res*, **64**, 1757-64.
- Xu, H.E., Stanley, T.B., Montana, V.G., Lambert, M.H., Shearer, B.G., Cobb, J.E., McKee, D.D., Galardi, C.M., Plunket, K.D., Nolte, R.T., Parks, D.J., Moore, J.T., Klierer, S.A., Willson, T.M. and Stimmel, J.B. (2002) Structural basis for antagonist-mediated recruitment of nuclear co-repressors by PPARalpha. *Nature*, **415**, 813-7.
- Yang-Yen, H.F., Chambard, J.C., Sun, Y.L., Smeal, T., Schmidt, T.J., Drouin, J. and Karin, M. (1990) Transcriptional interference between c-Jun and the glucocorticoid receptor: mutual inhibition of DNA binding due to direct protein-protein interaction. *Cell*, **62**, 1205-15.
- Yoon, J.C., Puigserver, P., Chen, G., Donovan, J., Wu, Z., Rhee, J., Adelmant, G., Stafford, J., Kahn, C.R., Granner, D.K., Newgard, C.B. and Spiegelman, B.M. (2001) Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1. *Nature*, **413**, 131-8.
- Zhong, H., SuYang, H., Erdjument-Bromage, H., Tempst, P. and Ghosh, S. (1997) The transcriptional activity of NF-kappaB is regulated by the IkappaB-associated PKAc subunit through a cyclic AMP-independent mechanism. *Cell*, **89**, 413-24.
- Zhong, H., Voll, R.E. and 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**, 661-71.
- Zhu, X.Y., Liu, Y.J., Lu, J. and Xu, R.B. (2004) Knockdown of glucocorticoid receptor expression by RNA interference promotes cell proliferation in murine macrophage RAW264.7 cells. *J Steroid Biochem Mol Biol*, **92**, 375-82. Epub 2004 Dec 31.
- Zilliacus, J., Wright, A.P., Carlstedt-Duke, J. and Gustafsson, J.Å. (1995) Structural determinants of DNA-binding specificity by steroid receptors. *Mol Endocrinol*, **9**, 389-400.