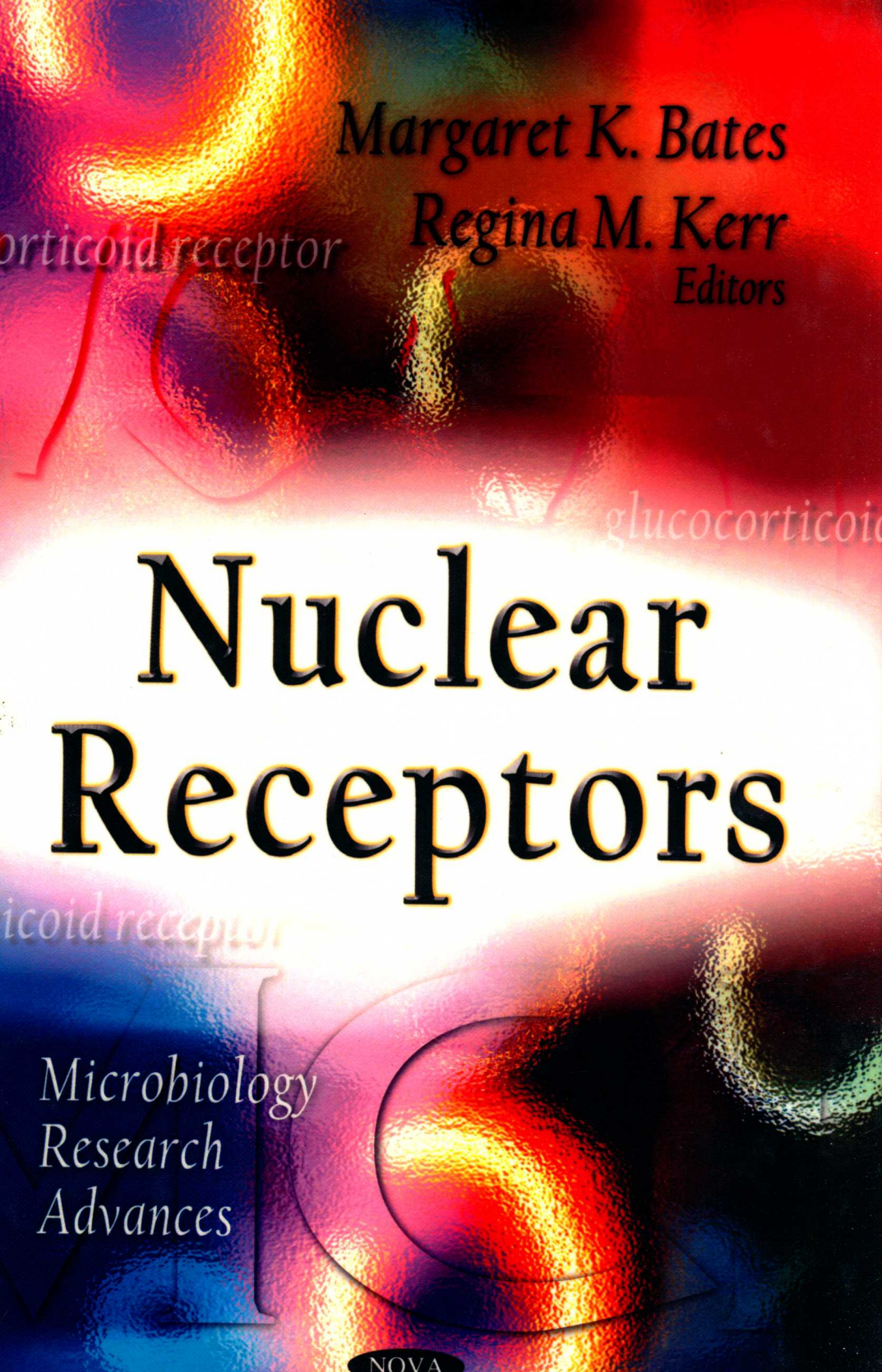
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Editors
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NUCLEAR RECEPTORS

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PREFACE

Nuclear receptors are involved in various aspects of intracellular signal transduction within a range of tissues and play an important role as regulators in numerous essential biological functions. In this new book, the authors present topical research in the study of nuclear receptors, including glucocorticoid receptor signaling in cardiovascular disease; steroid receptor coactivators and endocrine treatment in breast cancer; the effects of nitric oxide on nuclear receptors as a tool for studying gene regulation; vitamin K as a ligand of steroid and xenobiotic receptors and androgen receptors and prostate cancer.

Chapter 1 – Glucocorticoid administration has been considered for the reduction of atherosclerosis and restenosis following percutaneous coronary intervention. Excess of glucocorticoids (GCs), due to either chronic exogenous treatment or to endogenous hypersecretion of cortisol (such as depression, systemic inflammation and Cushing disease) has been associated with increased cardiovascular risk. The GC receptor is the cornerstone molecule mediating GCs' cellular effects. Myocyte sensitivity to GCs depends on intracellular, pre-receptor metabolism of active cortisol to inactive cortisone by the enzyme 11β-hydroxy steroid dehydrogenase. The molecular basis of GC-induced cardiovascular effects such as atherosclerosis and hypertension remain elusive. Toll-like receptors (TLRs) represent the first line of host defense against microbial infection and play a significant role in both innate and adaptive immunity by recognition of exogenous pathogen-associated molecular patterns and endogenous ligands. The TLR and GR signaling pathways interact and modulate the inflammatory response. Innate immune and inflammatory pathways have been implicated in cardiac dysfunction after global myocardial ischemia. There are 10 TLRs identified to date and they

bind to a variety of pathogenic agents such as lipopeptide (TLR2) and lipopolysaccharide (TLR4) by molecular pattern recognition. TLR4 is expressed in myocardial cells and increased expression of this receptor is observed in cardiac myocytes from human and animal models of ischemic cardiac injury. Stimulation of TLRs leads to the activation of various downstream transcription factors in particular nuclear factor (NF)-κB and the production of inflammatory cytokines in myocardial cells. These cytokines in turn activate TLRs in a positive feedback mechanism that sustains inflammation thereby contributing to disease progression. S100B, a member of the S100 family of calcium-binding proteins is induced by adrenergic stimulation in myocardial cells following ischemic cardiac injury and depending on the concentration achieved, via receptor for advanced ligation endproducts (RAGE) ligation, results in activation of NF-κB and trophic or apoptotic cell responses. Preliminary data from hypoxic myocardial cells demonstrate a possible association between TLR4 and S100B. The common signaling pathway involves the activation of NF-κB leading to GC receptor activation and transrepression of target genes. In this chapter we provide a comprehensive review of GC receptor signaling and its convergence with TLR4- and RAGE-S100B dependent signaling pathways as it relates to inflammation and the progression of cardiovascular disease.

Chapter 2 – Breast cancer is the most frequent malignancy in women in the Western world. Hormone receptor positive breast cancers are managed with endocrine treatment in which the estrogen receptor (ER) is blocked using a selective estrogen receptor modulator (SERM) such as tamoxifen or by targeting the estrogen synthesis using aromatase inhibitors (AIs). Nuclear receptor coactivators have been pointed out as the main determinants of tissue-, cell- and promoter specific effects of tamoxifen, and they are important regulators of ER mediated gene transcription under estrogen deprivation induced by aromatase inhibition.

The steroid receptor coactivator (SRC) family comprises SRC-1, SRC-2/TIF-2 and SRC-3/AIB1. Typically they enhance the transcriptional activity of ligand-bound ER by binding to the activation function-2 (AF-2) pocket, and recruit the basal transcription factors and chromatin-remodeling complex, acetyltransferase proteins, methyltransferases and ubiquitin ligases. 4-hydroxytamoxifen works as an ER antagonist by binding to the nuclear receptor and inducing a displacement of helix 12 that blocks binding of coactivators and favors corepressor recruitment. However, high levels of coactivators relative to corepressors may force ER into an active structural conformation where 4-hydroxytamoxifen leads to ER agonistic effects via AF-

1 by indirect binding to DNA and recruitment of coactivators. While SRC-1 and SRC-2/TIF-2 are expressed in normal and malignant breast tissue, SRC-3/AIB1 predominates with overexpression in >30% and gene amplification in 5 – 10% of breast cancers. Our recent studies in human breast cancer have shown that treatment with tamoxifen or AIs enhances gene expression of the SRCs. Others have reported that high levels of SRC-1 are associated with nodal involvement and resistance to endocrine treatment. SRC-3/AIB1 and the growth factor receptor HER-2/neu are often coexpressed in breast cancers, and poor response to tamoxifen treatment and reduced disease-free survival are found when tumors overexpress SRC-1 or SRC-3/AIB1 together with HER-2/neu. The SRCs are regulated by post-translational modifications by for instance mitogen activated protein kinases (MAPKs) which operate downstream of HER-2/neu and stabilize and functionally activate SRC proteins, a mechanism which could contribute not only to tamoxifen resistance, but also to estrogen hypersensitivity and resistance to AIs. In summary, steroid receptor coactivators are crucial in ER regulated gene transcription. Accumulated evidence points to an association between coactivator levels, effect of and response to endocrine treatment and long-term outcome in human breast cancer. The SRCs are involved in crosstalk between ER and growth factor pathways that are activated in breast cancer, making coactivators important in breast cancer development and interesting as potential therapeutic targets.

Chapter 3 – Nuclear receptors are involved in various aspects of intracellular signal transduction on a range of tissue and play an important role as regulators in numerous essential biological functions. In the thymus, these nuclear receptors also participate in positive or negative selection during T cell development.

In particular, the glucocorticoid receptor (Gr) and Nur77 play central role s in apoptosis induction mediated by the T cell receptor (TCR) in mature thymocytes or glucocorticoids (GCs) in immature thymocytes, respectively. Recently, we demonstrated that death-associated protein 3 (DAP3) was critical for TCR-mediated induction of apoptosis downstream of Nur77 in immature thymocytes. The DAP3 is an evolutionarily conserved GTP binding protein that plays a number of roles in normal mitochondrial physiology and in apoptosis induced via the tumor necrosis factor (TNF) family of death receptors. This chapter reviews recent studies of the signal transduction mediated by Gr and Nur77 in thymocyte development, focusing on signaling molecules, such as DAP3, involved in the signaling pathways of Gr or Nur77. Briefly, discussion which have attracted attention are summarized as follows:

(1). signaling molecules interacting with Gr or Nur77, (2). the functional role of Gr or Nur77 in subcellular localization, (3). the function of genes subject to induction by Gr or Nur77, (4). crosstalk and its physiological importance in the signal transduction mediated by Gr and Nur77.

Chapter 4 – Nitric oxide (NO), a free radical gas, is an omnipresent intercellular messenger in all vertebrates. Originally described as a cardiovascular signal molecule NO elicites a variety of physiological functionslike muscle contractility, platelet aggregation, metabolism, neuronal activity, and immune responses in a broad range of tissues.

The molecule originates from the action of nitric oxide synthases (NOS) which are either induced (iNOS) or constitutively expressed (eNOS, nNOS). The underlying mechanisms of NO action are primarily an elevation of guanosine 3',5'-cyclic monophosphate due to the stimulation of soluble guanylyl cyclase, inhibition of mitochondria respiration and nitrosylation of proteins.

In vitro, NO has been shown to modulate the activity of a variety of nuclear receptors (NR) of the steroid receptor superfamily like the estrogen (ER) or the androgen receptor (AR).

Nuclear receptors are transcription factors characterized by a ligand binding domain, a highly conserved DNA binding domain consisting of two Cys4-type zinc fingers and a transactivation domain, possessing one of the two activation function domains.

Upon ligand binding, NR predominantly act via binding to so called hormone response elements on the DNA thus regulating gene expression. Essential features which regulate the activity and function of NR are receptor concentration, post translational modifications like phosphorylation or acetylation which trigger receptor dimerization, nucleocytoplasmic shuttling or binding of comodulators.

Despite their different modes of action, the signaling pathways of nitric oxide and NR interfere ~~a~~ manifold. Estrogen and progesterone are known to up-regulate NO synthesis whereas glucocorticoids and progesterone decrease NO bioavailability. Due to its unique physicochemical properties (high reactivity of NO-radicals, short half-life, excellent membrane permeability) the NO-molecule is also able to directly interact with nuclear receptors thereby blocking their activity.

In the chapter, the authors will summarize the so far known effects of NO on nuclear receptors and demonstrate its potential use as a tool for studying gene regulation. In addition they will discuss the physiological relevance of NO/NR interaction.

Chapter 5 – Vitamin K is a fat-soluble vitamin essential for blood coagulation. Natural vitamin K includes vitamin K1 (phylloquinone), present mainly in vegetables, and vitamin K2 (menaquinone), which is synthesized by microorganisms and is present in food such as natto (fermented soy beans). Vitamin K1 is converted to vitamin K2, the functionally active form, in the body.

Vitamin K was shown to play an essential role in the hepatocytes by maintaining the activity of coagulation factors II, VII, IX, and X and of anticoagulants, protein C, and protein S. Recently, extrahepatic actions of vitamin K have also been reported.

The administration of vitamin K was shown to prevent bone fracture, and this led to its clinical application in cases of osteoporosis in East Asian countries. Epidemiological studies have shown that the lack of vitamin K causes osteoarthritis and imposes a risk of coronary artery disease. Some clinical studies have suggested its antitumor activity in cases of hepatocellular carcinoma and other cancers.

Vitamin K is a natural and safe nutrient. The novel effect of vitamin K that involves the nuclear receptor SXR/PXR has been elucidated. It can be presumed that this novel effect exists in other organs and tissues where SXR/PXR expression is detected, like in the bone and in the liver.

Further investigation on this mechanism is warranted to understand the precise function of vitamin K and the pathology of the concerning diseases, and to discover new therapeutic approaches for these diseases.

Chapter 6 – Nuclear receptors are a class of proteins that have the ability to directly bind to DNA and regulate gene expression, and these receptors are classified as transcription factors. This report focuses on a new function of AR (androgen receptor). Androgen receptors (ARs) belong to the steroid receptor family and play an essential role in the generation and development of the prostate. Androgen receptors have similar conserved domains that are composed of an NTD (N-terminal domain), a DBD (DNA-binding domain), and an LBD (ligand-binding domain). The NTD works stabilize bound androgen and the AR-LBD mediates the interaction between AR and other proteins, which include Hsps (heat-shock proteins). In the absence of androgen, AR remains in the cytoplasm in an inactive form. After AR binds to androgens, activated AR can bind with other signal molecules and form functional complexes. Then, the complex translocates to the nucleus and regulates the gene expression for androgen regulated genes. Recently, some research has shown that AR can interact with DDC (L-dopa decarboxylase), a key molecule for serotonin (5-HT) synthesizing. Serotonin is a well known

neurotransmitter but has been mentioned in the relationship with the generation of the prostate. Then, we introduce here that AR can regulate prostate cancer progression via the serotonin synthesis process.

A suggested rewrite of the previous sentence, placed here to avoid ambiguity: This chapter suggests that AR may play a role in regulating the progress of prostate cancer via the serotonin synthesis process.

Chapter 7 – Plants are immobile and, therefore, confronted with a variety of environmental stresses. To adapt to these harmful conditions, plants have developed effective and complex stress signal transduction pathways between the nucleus and cytoplasm for response to and survival from stress conditions. In eukaryotic cells, the nuclear envelope separates the cytoplasm from the nucleus, and the nuclear pore complex (NPC) is the gateway for signal molecules trafficking across the nuclear envelope. Small molecules utilize passive diffusion to pass through the NPC; however, the efficient and directed translocation of macromolecules requires nuclear transport receptors to facilitate the passage through the NPC. In this regard, importinβ-like nuclear transport receptors are the main receptors for nuclear transport in Arabidopsis. Some of these receptors act as nuclear import receptors (importins) and some as nuclear export receptors (exportins). In addition, many stress responses in plants are controlled by the nucleocytoplasmic partitioning of regulatory molecules between the cytoplasm and nucleoplasm, such as light, temperature, and responses to cytokinin-signal transduction and pathogen infection. Thus, importins and exportins are very important for plant growth, development, and stress response.

Chapter 8 – Peroxisome proliferator-activated receptors (PPARs) are ligand-activated nuclear receptors that regulate gene expression and are modulated by interaction with corepressors and coactivators. Natural and synthetic ligands promote heterodimerization of PPARs with the retinoid-X-receptor (RXR), facilitating their binding to consensus DNA sequences on target genes. To date, three subtypes of PPAR have been identified – α, δ, and γ; with the γ subtype consisting of two distinct functional isoforms, γ1 and γ2. Although structurally similar, the PPAR subtypes have specific tissue distribution and functions. PPARs regulate multiple cellular functions, such as cell proliferation, the immune response and lipid metabolism and therefore their ligands have been investigated for their potential use in various clinical settings. For example, the PPARγ ligands comprising the thiazolidinedione class of drugs have been used for the management of type 2diabetes. The potential use of PPAR ligands in autoimmune diseases is also being investigated whereas the specific role of PPARs in tumor development is still

controversial. Despite some drawbacks, PPARs still remain as potential therapeutic targets for various conditions and currently dual and pan agonists are being investigated for this purpose. Taken together, the role of PPARs in various cellular processes and disease pathogenesis still requires further investigation and continues to be an exciting field of research. This review will attempt to provide examples of some of the recent findings in these areas of research, highlighting the mechanisms of action and the potential use of PPAR agonists as well as the challenges that still need to be addressed.

CONTENTS

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Chapter 1

GLUCOCORTICOID RECEPTOR SIGNALING AND ITS POTENTIAL CONVERGENCE WITH TOLL-LIKE RECEPTOR (TLR)-4 AND RECEPTOR FOR ADVANCED GLYCATION END-PRODUCTS (RAGE) SIGNALING PATHWAYS IN CARDIOVASCULAR DISEASE

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ABSTRACT

Glucocorticoid administration has been considered for the reduction of atherosclerosis and restenosis following percutaneous coronary intervention.

Excess of glucocorticoids (GCs), due to either chronic exogenous treatment or to endogenous hypersecretion of cortisol (such as depression, systemic inflammation and Cushing disease) has been associated with increased cardiovascular risk. The GC receptor is the cornerstone molecule mediating GCs' cellular effects. Myocyte sensitivity to GCs depends on intracellular, pre-receptor metabolism of active cortisol to inactive cortisone by the enzyme 11β-hydroxy steroid dehydrogenase. The molecular basis of GC-induced cardiovascular effects such as atherosclerosis and hypertension remain elusive. Toll-like receptors (TLRs) represent the first line of host defense against microbial infection and play a significant role in both innate and adaptive immunity by recognition of exogenous pathogen-associated molecular patterns and endogenous ligands. The TLR and GR signaling pathways interact and modulate the inflammatory response. Innate immune and inflammatory pathways have been implicated in cardiac dysfunction after global myocardial ischemia. There are 10 TLRs identified to date and they bind to a variety of pathogenic agents such as lipopeptide (TLR2) and lipopolysaccharide (TLR4) by molecular pattern recognition. TLR4 is expressed in myocardial cells and increased expression of this receptor is observed in cardiac myocytes from human and animal models of ischemic cardiac injury. Stimulation of TLRs leads to the activation of various downstream transcription factors in particular nuclear factor (NF)-κB and the production of inflammatory cytokines in myocardial cells. These cytokines in turn activate TLRs in a positive feedback mechanism that sustains inflammation thereby contributing to disease progression. S100B, a member of the S100 family of calcium-binding proteins is induced by adrenergic stimulation in myocardial cells following ischemic cardiac injury and depending on the concentration achieved, via receptor for advanced ligation endproducts (RAGE) ligation, results in activation of NF-κB and trophic or apoptotic cell responses. Preliminary data from hypoxic myocardial cells demonstrate a possible association between TLR4 and S100B. The common signaling pathway involves the activation of NF-κB leading to GC receptor activation and transrepression of target genes. In this chapter we provide a comprehensive review of GC receptor signaling and its convergence with TLR4- and RAGE-S100B dependent signaling pathways as it relates to inflammation and the progression of cardiovascular disease.

S100 Proteins and Rage Signaling

S100 Proteins Structure and Function

S100 proteins entail a multigenic family of calcium binding proteins of the EF- hand type (helix E-loop-helix F). These proteins are called S100 because of

their solubility in a 100% -saturated solution with ammonium sulphate at neutral pH. They are small acidic proteins, 10-12KDa and contain two distinct EF-hands, 4 α-helical segments, a central hinge region of variable length and the N-and C-terminal variable domains. Twenty five members of this family have been identified (Donato et al., 2009). Of these, 21 (S100A1-S100A18, trichohylin, filagrin and repetin) have genes clustered on a 1.6-Mbp segment of human chromosome 1 (1q21) while other members are found at chromosome loci 4q16 (S100P), 5q14 (S100Z), 21q22 (S100B), and Xp22 (S100G) (Engelkamp et al., 1993). S100 proteins are widely expressed in a variety of cell types and tissues. For example, S100A1 and S100A2 are found in the cytoplasm and nucleus of smooth-muscle cells of skeletal muscle, respectively (Donato 2001), S100P is located in the cytoplasm of placental tissue (Becker et al., 1992; Emoto et al., 1992) and S100B in cytoplasm of astrocytes of nervous system (Kligman and Marshak 1985). S100 proteins do not exhibit intrinsic catalytic activity but are calcium sensor proteins and through interaction with several intracellular effector proteins they contribute to the regulation of a broad range of functions such as contraction, motility, cell growth and differentiation, cell cycle progression, organization of membrane-associated cytoskeleton elements, cell survival, apoptosis, protein phosphorylation and secretion (Donato 1999, 2001; Donato et al 2009). For example, S100B regulates the cytoskeletal dynamics through disassembly of tubulin filaments, type III intermediate filaments (Donato et al., 2009) and binding to fibrillary proteins such as CapZ (Kilby et al., 1997) or inhibiting GFAP phosphorylation when stimulated by cAMP or calcium/calmodulin (Frizzo et al., 2004). To modulate these types of activities S100 proteins undergo conformational changes (Ikura 1996). Upon calcium binding the helices of S100 proteins rearrange, revealing a hydrophobic cleft, which forms the target protein binding site (Rustandi et al., 2000). Although target binding of S100 proteins is calcium-dependent, calcium independent interactions have been reported (Santamaria-Kisiel et al., 2006). Enzymes are the most common calcium independent target binding for the S100 proteins. S100B for example, interacts in a calcium-dependent manner with the cytoplasmic domain of myelin-associated glycoprotein and inhibits its phosphorylation by protein kinase (Kursula et al., 2000). It is also implicated in tau protein (Baudier and Cole 1988) and p53 phosphorylation (Markowitz et al., 2005), inhibition of Ndr kinase activity (Millward etal., 1998), and regulation of the activity of the GTPase Rac1 and Cdc42 effector IQGAP1 (Mbele et al., 2002). The most significant calcium-independent interactions of S100 proteins are their ability to bind to each other. They form homodimers, but heterodimerization adds to the complexity of this multiprotein family. Each subunit consists of two helix-loop-helix motifs