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Interactions in the transcriptional regulation exerted by Stat5 and by members of the steroid hormone receptor family ☆

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Abstract

The pathways which connect extracellular signals with the regulation of the activity of transcription factors are being investigated in molecular detail. Extensive progress has been made in the description of the mode of action of steroid hormones and of cytokines. Steroid hormones associate intracellularly with latent receptor molecules, cause the dissociation of masking proteins, the dimerization of receptors, and their binding to specific hormone response elements in the promoters of target genes. Cytokines also activate latent transcription factors (Stats—signal transducers and activators of transcription), but act through an enzymatic mechanism. Tyrosine kinases associated with the transmembrane cytokine receptors phosphorylate Stat molecules. The phosphorylated monomers dimerize and assume specific DNA binding ability. Both classes of transcription factors bind to different response elements and regulate different target genes and both signals, cytokines and steroid hormones, can affect growth differentiation and homeostasis of different cell types. Here, we describe that Stat5, a molecule activated by several essential cytokines, functionally interacts with members of the steroid receptor family. We find that glucocorticoid receptor, mineralocorticoid receptor and progesterone receptor synergize with Stat5 in the induction of the transcription from the β-casein gene promoter. The estrogen receptor diminishes Stat5 mediated induction and the androgen receptor has no effect. Conversely, Stat5 negatively interferes with glucocorticoid receptor, mineralocorticoid receptor and progesterone receptor induced transcription from the MMTV LTR and the estrogen receptor induced transcription from an ERE-containing promoter.

1. Introduction

Biological regulation is generally exerted through combinatorial events. Interaction of regulatory pathways and individual transcription factors results in cell type specific gene transcription. We have investigated the co-operation and interference between nuclear hormone receptors and Stat5.

Nuclear hormone receptors belong to a family of transcription factors dependent in their activity on hormonal ligands. The nuclear receptor family includes receptors for steroid hormones (estrogens, progestersons, glucocorticoids and mineralocorticoids) [1,2], receptors for thyroid hormone, retinoids, vitamin D3 and receptors whose ligands are still unknown (orphan receptors) [3]. Steroid hormones regulate essential biological phenotypes such as growth, differentiation and homeostasis. They function through the regulation of target genes in receptor expressing cell types. The steroid hormone receptors are characterized by a modular domain structure, with a carboxyl terminal ligand binding domain, a central DNA binding domain and an amino terminal transcriptional regulatory function [4–6]. It is thought that ligand association induces the dissociation of the receptor molecule from heat shock proteins, conformational changes which allow dimerization of the receptors and binding to DNA as well as the recruitment of coactivators. These are prerequisites for the enhancement of transcription of target genes. Steroid receptors bind as homodimers to hormone response elements which are composed of two hexanu-

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cleotide recognition sequences organized as inverted palindromes. Three nucleotides serve as spacers between the half sides.

Although different in their physiological functions, several steroid hormone receptors share a common response element. The DNA binding site, initially found for the glucocorticoid receptor, is a 15 bp imperfect palindromic DNA sequence present in the promoter region of target genes. Four copies of the GRE have been found in the glucocorticoid inducible mouse mammary tumor virus LTR. The same DNA sequences can also confer transcriptional induction through the progesterone, androgen and mineralocorticoid receptors. The nucleotide sequences defining a progesterone response element, androgen response element or mineralocorticoid response element are identical to the glucocorticoid response element. A different situation exists for the estrogen receptor and the estrogen response element comprises a 13 bp perfect palindromic DNA sequence which is clearly distinct from the glucocorticoid response element [7]. Although additional components, such as coactivators and basal transcription factors are required for transcriptional induction, provision of the steroid receptors and a reporter indicator gene allows the study of steroid hormone action in transfected COS cells.

Stat proteins (signal transducers and activators of transcription) mediate the response to cytokines and growth factors and are comparable but distinct in their mode of action to steroid hormone receptors. Cytokines do not enter the cells but activate cell surface receptors and associated tyrosine kinases which in turn cause the tyrosine phosphorylation of Stat proteins [8,9]. Phosphorylated Stat proteins dimerize, translocate to the nucleus and assume the ability to bind to specific DNA sequences in target gene promoters. Stat proteins bind as dimers to DNA target sites, comprising a 9 bp consensus sequence. This sequence is fundamentally different from the one to which nuclear hormone receptors bind [10–12]. Distinct functional domains have been identified within Stat molecules. An amino terminal domain mediates cooperativity between Stat molecules, a central domain confers the DNA binding specificity of the dimer and a SH-2 domain mediates dimerization through phosphotyrosine recognition. The carboxyl terminus comprises a transactivation domain [13].

The study of differentiation specific gene expression in mammary epithelial cells has provided first insights into the molecular mechanisms governing the co-operation between steroid hormone receptors and a Stat mediated pathway [14–20]. Prolactin and glucocorticoid hormones co-operate in the regulation of milk protein gene transcription and a novel role for the glucocorticoid receptor has been defined. The glucocorticoid receptor acts as a transcriptional coactivator for Stat5 and enhances Stat5 dependent transcription of the β-casein gene promoter. This action of the glucocorticoid receptor is dependent on the complex formation with Stat5 and recruitment to the β-casein gene promoter, but independent of a GRE. The complex formation between Stat5 and the glucocorticoid receptor diverts the glucocorticoid receptor from GRE containing promoters and diminishes the glucocorticoid response of for example, MMTV LTR transcription [21,22].

We have extended our investigations and analysed functional interactions between Stat5 and the mineralocorticoid receptor, progesterone receptor, androgen receptor and estrogen receptor [23]. We find that the mineralocorticoid receptor and the progesterone receptor synergize with Stat5 in the induction of the β-casein gene promoter, although to a smaller extent than the glucocorticoid receptor. Activation of the estrogen receptor diminishes Stat5 dependent induction, whereas the androgen receptor has no effect. The inducibility of glucocorticoid, mineralocorticoid and progesterone responsive genes is diminished by the simultaneous activation of Stat5. The same is true for an estrogen receptor dependent indicator gene.

2. Material and methods

2.1. Plasmids

The luciferase constructs for the β-casein gene promoter (-344 to -1) and the MMTV LTR gene promoter have been described previously [8,9,21] as the expression vectors for MGF-Stat5 (pXM-MGF), the long form of the Prl R (pcDNA1-PrlR) and the human glucocorticoid receptor pRSVhGRα (GR) [21]. The mineralocorticoid receptor expression vector pRSVhMR (MR) was provided by A. Cato, Karlsruhe [24]. The estrogen responsive element (ERE) luciferase reporter construct, pGL2-ERE tk-luc, was obtained from M. Parker, London. The expression vector for the human estrogen receptor (pRSVhER) and the human progesterone receptor (hPR B) in the expression vector pSG5 were provided by P. Chambon, Strasbourg, France [25]. The androgen receptor (ARO) in the pSV expression plasmid was obtained from A. O. Brinkmann, Rotterdam [26].

2.2. Cell culture and transfection

COS7 cells were routinely maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum, 2 mM glutamine and 50 μg/ml gentamycin. Transfection experiments were performed using the calcium phosphate precipitation technique [9]. Half-confluent COS7 cells in 10 cm dishes were

cotransfected with expression vectors for Stat5 (2.5 μg), the prolactin receptor (2 μg), the glucocorticoid receptor (2 μg) or another member of the steroid hormone receptor family (the mineralocorticoid receptor (2μg), the progesterone receptor B (5μg), the androgen receptor (10μg) or the estrogen receptor (2μg)). Five micrograms of a luciferase reporter construct (either β-casein-luc or MMTV LTR luc or ERE tk luc) was included along with 2 μg of pH 110 encoding the β-galactosidase gene driven by the SV40-promoter, to monitor transfection efficiency. The DNA was adjusted to 20 μg with empty vector-DNA. One day after transfection, hormone induction was performed with 5 μg/ml ovine prolactin and/or 10^{-7} M dexamethasone or progesterone R5020 10^{-7} M or androgen R 1881 10^{-9} M or estrogen 10^{-7} M for 16 h.

2.3. Luciferase and β-galactosidase assays

Two days after transfection cells were harvested, washed twice in cold phosphate-buffered saline (PBS) and lysed in 25 mM glycylglycine pH 7.8, 1 mM DTT, 15 mM $MgSO_4$, 4 mM EGTA and 1% Triton for 10 min at 4°C. Samples were centrifuged for 5 min at 14,000 rpm. Cleared supernatants were used for luciferase and β-galactosidase assays. For β-galactosidase determination, 20 μl of extracts were added to 200 μl of reaction buffer containing 100 mM Na-phosphate pH 8.0, 1 mM MgCl and 1 × Galacton (Tropix) and incubated for 30 min at room temperature. Measurements were made by injecting 300 μl of accelerator solution (10% Emerald luminescent amplifier and 0.2 N NaOH) and the samples were counted for 20 s in the Luminometer 953 (Berthold). Luciferase activities (100 μl extracts and 300 μl of accelerator solution) were quantified in the same apparatus and normalized to the β-galactosidase activities.

3. Results

3.1. Glucocorticoid receptor activation results in a strong, mineralocorticoid receptor activation in a slight enhancement of Stat5-dependent transcription from the β-casein gene promoter

The transfection of receptor molecules, signaling components and indicator genes into COS cells has proven to be a valuable asset in the study of signaling pathways. COS cells do not express appreciable amounts of Stat5 or members of the steroid receptor family. The signaling pathways for steroid hormones and cytokines, however, can be reconstituted by providing these components in transient transfection assays.

When COS cells were transiently transfected with

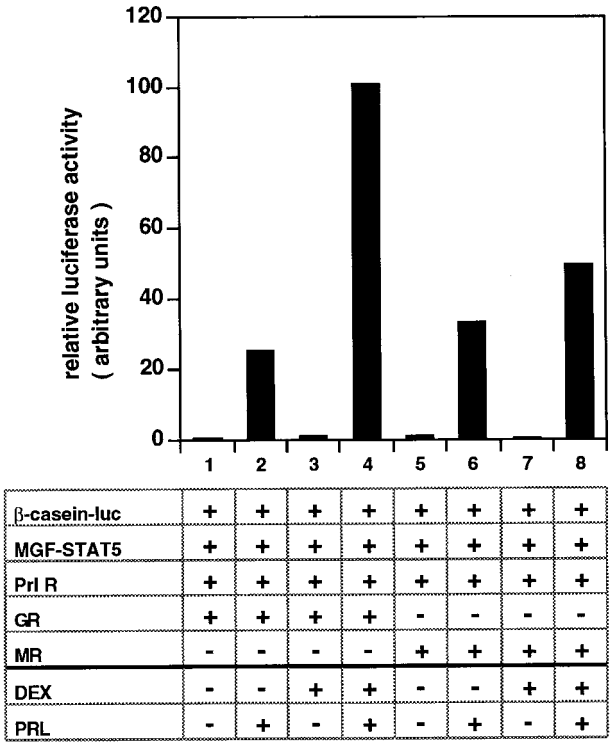
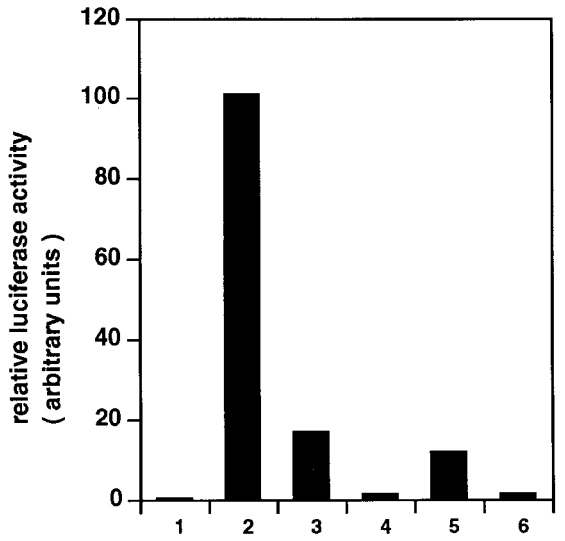


Fig. 1. Stat5 induction of the β-casein gene promoter is strongly enhanced by glucocorticoid receptor and slightly enhanced by mineralocorticoid receptor activation. COS7 cells were transiently cotransfected with constructs encoding Stat5 (MGF-Stat5), the prolactin receptor (Prl R), the glucocorticoid receptor (GR) (lanes 1 to 4) or the mineralocorticoid receptor (MR) (lanes 5 to 8), the β-casein-luciferase reporter gene (β-casein-luc) and an expression plasmid encoding β-galactosidase to monitor transfection efficiency. Luciferase activities were determined in extracts from cells induced with hormones indicated for 16 h and normalized to the β-galactosidase activities as described in Materials and methods.

the prolactin receptor, Stat5 and a β-casein promoter luciferase indicator gene, treatment of the transfected cells with prolactin resulted in enhanced luciferase activity (Fig. 1, lanes 1 and 2). When in addition the glucocorticoid receptor was provided, a synergistic action of the glucocorticoid receptor and Stat5 could be observed upon treatment of the cells with dexamethasone and prolactin (lane 4). Dexamethasone by itself had no effect (lane 3). When instead of the glucocorticoid receptor the mineralocorticoid receptor was cointroduced into these cells enhancement of transcription upon treatment with prolactin was observed (lane 6) and a slight synergism resulted upon treatment of the cells with dexamethasone and prolactin (lane 8). These observations indicate that similarly to the glucocorticoid receptor, the mineralocorticoid receptor is able to synergize with Stat5 in the induction of the β-casein promoter, although to a lesser extent.

We also investigated the activation of the glucocorticoid receptor and the mineralocorticoid receptor in transfected COS cells and their effect on the transcrip-



	1	2	3	4	5	6
MMTV/LTR- luc	+	+	+	+	+	+
MGF-STAT5	+	+	+	+	+	+
Prl R	+	+	+	+	+	+
GR	+	+	+	-	-	-
MR	-	-	-	+	+	+
DEX	-	+	+	-	+	+
PRL	-	-	+	-	-	+

Fig. 2. Activation of Stat5 represses the mineralocorticoid receptor induction of the MMTV LTR gene promoter. COS7 cells were transiently cotransfected with constructs encoding Stat5 (MGF-Stat5), the prolactin receptor (Prl R), the glucocorticoid receptor (GR) (lanes 1 to 3) or the mineralocorticoid receptor (MR) (lanes 4 to 6), the MMTV-luciferase reporter gene (MMTV LTR-luc) and an expression plasmid encoding β-galactosidase to monitor transfection efficiency. Luciferase activities were determined in extracts from cells induced with hormones indicated for 16 h and normalized to the β-galactosidase activities.

tion of a luciferase gene under the control of the MMTV LTR. The MMTV LTR contains all glucocorticoid response elements. Transfection of COS cells with the glucocorticoid receptor and the MMTV LTR luciferase gene and treatment of the transfected cells with dexamethasone results in a strong induction of luciferase activity (Fig. 2, lanes 1 and 2). However, simultaneous activation of Stat5 through prolactin and the prolactin receptor diminishes the glucocorticoid receptor mediated effect on MMTV LTR induction (lane 3). We investigated, if the mineralocorticoid receptor would be similarly affected. Treatment of cells transfected with the mineralocorticoid receptor and activation of this receptor resulted in an induction of luciferase activity (lanes 4 and 5). This activity is much lower than the one obtained upon glucocorticoid receptor activation (lane 2). Nevertheless, simultaneous activation of the mineralocorticoid receptor and Stat5 (lane 6) resulted in a strongly diminished mineralocorticoid receptor response. The activation of Stat5 negatively interferes with the ability of the mineralocorticoid receptor to induce gene transcription.

3.2. Progesterone receptor activation results in an enhancement of Stat5 dependent transcription on the β-casein gene promoter

We also investigated if the activation of the progesterone receptor would have functional consequences for the extent of Stat5 mediated induction of the β-casein luciferase indicator gene. Introduction of the progesterone receptor into COS cells and the simultaneous activation of the progesterone receptor through R5020 and the prolactin receptor through prolactin causes a stronger induction of luciferase activity (Fig. 3, lane 11) than the treatment of the cells with prolactin only (lanes 9). Progesterone treatment by itself has no effect on the utilization of the β-casein gene promoter (lanes 8 and 10). Although progesterone synergizes with prolactin in the induction of the β-casein gene promoter, the absolute values of luciferase activity observed are much lower when compared to the synergism obtained with dexamethasone (compare lanes 3 and 11, notice the different scales). Both steroid hormones, dexamethasone and R5020, positively synergize with prolactin in the induction of the indicator gene. The simultaneous provision of dexamethasone and R5020 does not cause a further enhancement of transcription (lane 5). The synergism observed is not additive.

We also tested the effect of Stat5 on a progesterone inducible indicator gene. For this purpose, the MMTV LTR luciferase activity was measured upon induction of transfected cells with R5020 alone or upon treatment with prolactin and R5020 (Fig. 4, lanes 7 and 8). Progesterone receptor activation caused an induction of luciferase activity (lanes 5 and 7). This induction of activity was diminished by the simultaneous activation of Stat5 through prolactin (lane 8). Although the effect of the activation of Stat5 is qualitatively similar on the induction mediated by glucocorticoid receptor and progesterone receptor, the absolute luciferase values have to be taken into consideration (notice the two different scales in Fig. 4).

3.3. Activation of the androgen receptor does not result in co-operation with Stat5 in the induction of the β-casein gene promoter

We tested a third member of the steroid receptor family with respect to its potential to co-operate with Stat5. For this purpose the androgen receptor was co-introduced with the prolactin receptor, Stat5 and the β-casein luciferase indicator gene into COS cells (Fig.

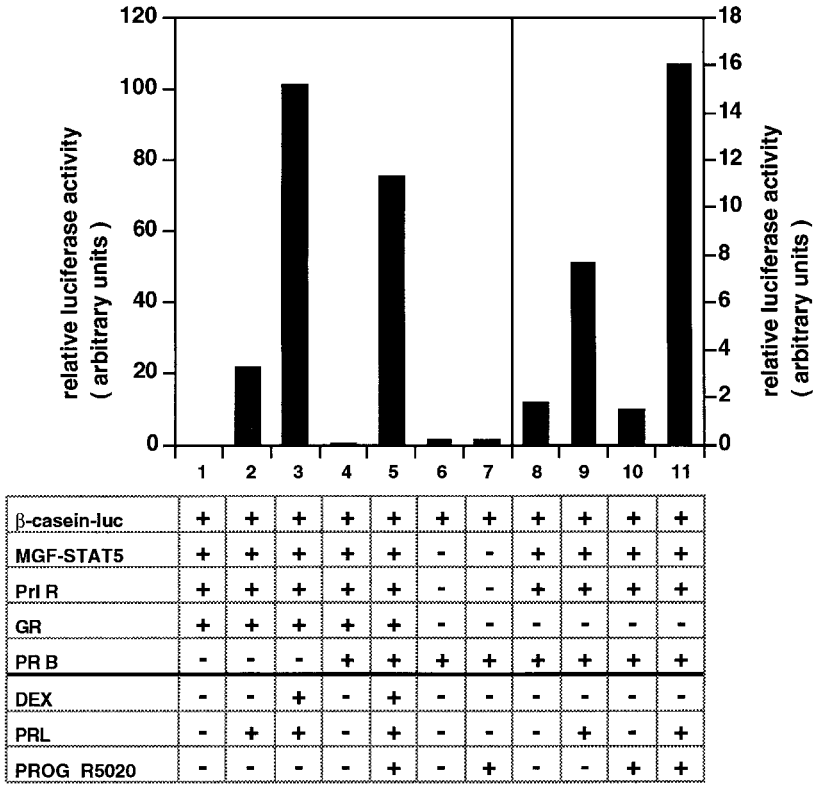


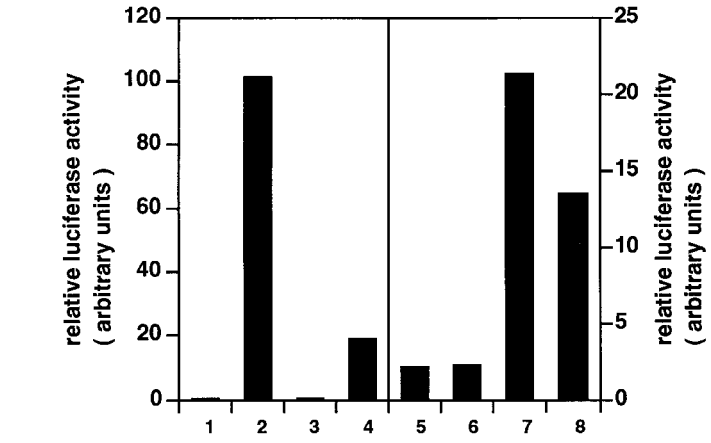
Fig. 3. Stat5 induction of the β-casein gene promoter is enhanced by progesterone receptor activation. COS7 cells were transiently cotransfected with constructs encoding Stat5 (MGF-Stat5), the prolactin receptor (Prl R), the glucocorticoid receptor (GR) (lanes 1 to 5) or the progesterone receptor (PRB) (lanes 4 to 11), the β-casein-luciferase reporter gene (β-casein-luc) and an expression plasmid encoding β-galactosidase to monitor transfection efficiency. Luciferase activities were determined in extracts from cells induced with hormones indicated for 16 h and normalized to the β-galactosidase activities.

5). Although the expression of the androgen receptor did not interfere with the prolactin induced enhancement of luciferase activity (lanes 5 and 7) no synergism between Stat5 and the androgen receptor could be observed (lane 7). For purposes of comparison the induction with prolactin or prolactin and dexamethasone are shown in lanes 1 to 3. The simultaneous activation of the androgen receptor and the glucocorticoid receptor (lane 9) seemed to negatively effect the synergism between Stat5 and the glucocorticoid receptor.

3.4. Activation of the estrogen receptor suppresses Stat5 mediated transcriptional induction of the β-casein gene promoter

The DNA sequence recognized by the estrogen receptor differs from the one shared by the glucocorticoid, mineralocorticoid, androgen and progesterone receptors. Since we have shown previously that the specific DNA recognition function of the glucocorticoid receptor is not required for the co-operation with Stat5, we also tested the effect of the estrogen receptor on Stat5 mediated transcriptional induction. Cells transfected with the prolactin receptor, Stat5, the glucocorticoid receptor and the β-casein luciferase indicator gene were inducible with prolactin alone (Fig. 6, lane 5) and stronger inducible with dexamethasone and prolactin (lane 2). Activation of the estrogen receptor did not synergize with Stat5. On the contrary, prolactin induced luciferase activity was strongly diminished through estrogen receptor activation (lane 6).

An estrogen responsive indicator gene was introduced into the COS cells. This indicator gene comprises a synthetic promoter element in which an estrogen response element is linked to a minimal tk promoter (Fig. 7). Upon treatment of the COS cells transfected with the estrogen receptor, the ERE-tk-luc with estrogen, a strong enhancement of luciferase activity results (lanes 1 and 2). Activation of the prolactin receptor through prolactin does not interfere with this induction in the absence of Stat5 (lanes 3 and 4). However, increasing amounts of Stat5 (lanes 5 to 9) negatively interfere with the estrogen receptor mediated induction of the indicator gene. This result identifies Stat5 as a negative regulator of estrogen mediated gene transcription.



MMTV/LTR-luc	+	+	+	+	+	+	+	+
MGF-STAT5	-	-	+	+	+	+	+	+
Prl R	-	-	+	+	+	+	+	+
GR	+	+	+	+	-	-	-	-
P R B	-	-	-	-	+	+	+	+
DEX	-	+	-	+	-	-	-	-
PRL	-	-	-	+	-	+	-	+
PROG R5020	-	-	-	-	-	-	+	+

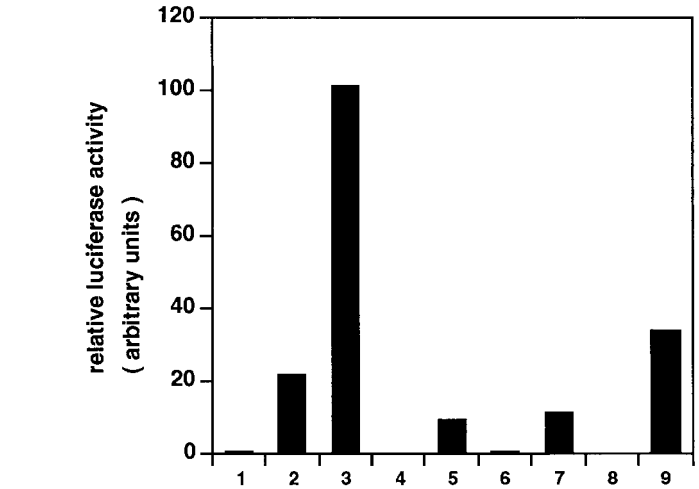
Fig. 4. Activation of Stat5 slightly represses the progesterone receptor induction of the MMTV LTR gene promoter. COS7 cells were transiently cotransfected with constructs encoding Stat5 (MGF-Stat5) (lanes 3 to 8), the prolactin receptor (Prl R) (lanes 3 to 8), the glucocorticoid receptor (GR) (lanes 1 to 4) or the progesterone receptor (PRB) (lanes 5 to 8), the MMTV-luciferase reporter gene (MMTV LTR-luc) and an expression plasmid encoding β-galactosidase to monitor transfection efficiency. Luciferase activities were determined in extracts from cells induced with hormones indicated for 16 hrs and normalized to the β-galactosidase activities.

4. Discussion

The molecular description of signaling pathways has yielded substantial insights into the mechanisms by which extracellular signals modulate gene transcription. Receptor mediated events result in the activation of latent transcription factors which allows them to bind to promoter sequences of particular target genes. Especially the description of steroid hormone action and cytokine action has yielded a high resolution.

Crystallization studies have shown how steroid hormone receptors and Stat proteins interact with DNA and how the specificity of binding for specific response elements is achieved [27,28]. Less is known how these protein DNA complexes communicate with the preinitiation complex which contains RNA polymerase II and the general transcription factors required for basal transcription [29]. It is commonly assumed that transcription factors with specific DNA binding ability couple to the preinitiation complex through intermediary proteins, coactivators [30,31]. These coactivators are thought to function as a bridge between the activated transcription factor and the basal transcription machinery [32]. Coactivators normally do not contain a specific DNA binding domain, they are recruited to the initiation complex by interactions with different protein partners [33,34]. They are thought not to play a role in basal transcription but are required for transcriptional enhancement induced by sequence specific transcription factors. Some of them encode histone acetyltransferase activity [35,36]. The family of p160 coactivators has been shown to mediate the response of steroid hormone receptors [37]. The p300/CBP transcription factor participates in the steroid hormone receptor response as well as in the Stat response [38–47].

Our experiments extend the concept of coactivators and corepressors [48–52] and show that even transcription factors with the ability to bind to specific DNA sequences can double as coactivators or corepressors. In the case of the glucocorticoid receptor we show that this receptor functions as a ligand dependent coactivator when the action of Stat5 on the level of the β-casein gene promoter is assayed. Similar observations have been made with Stat6. The specific DNA binding function of the glucocorticoid receptor is dispensible and the enhancing action is mediated via a direct protein-protein interaction. Conversely, the Stat5 molecule can be viewed as a corepressor of the glucocorticoid receptor. Binding of Stat5 to the glucocorticoid receptor most likely diverts the protein-protein complex from binding to a GRE and therefore prevents gluco-



	1	2	3	4	5	6	7	8	9
β-casein-luc	+	+	+	+	+	+	+	+	+
MGF-STAT5	+	+	+	+	+	+	+	+	+
Prl R	+	+	+	+	+	+	+	+	+
GR	+	+	+	-	-	-	-	+	+
ARO	-	-	-	+	+	+	+	+	+
DEX	-	-	+	-	-	-	-	-	+
PRL	-	+	+	-	+	-	+	-	+
ANDROGEN R1881	-	-	-	-	-	+	+		+

Fig. 5. Stat5 induction of the β-casein gene promoter does not cooperate with the androgen receptor. COS7 cells were transiently cotransfected with constructs encoding Stat5 (MGF-Stat5), the prolactin receptor (Prl R), the glucocorticoid receptor (GR) (lanes 1 to 3 and 8 to 9) or the androgen receptor (ARO) (lanes 4 to 9), the β-casein-luciferase reporter gene (β-casein-luc) and an expression plasmid encoding β-galactosidase to monitor transfection efficiency. Luciferase activities were determined in extracts from cells induced with hormones indicated for 16 h and normalized to the β-galactosidase activities.

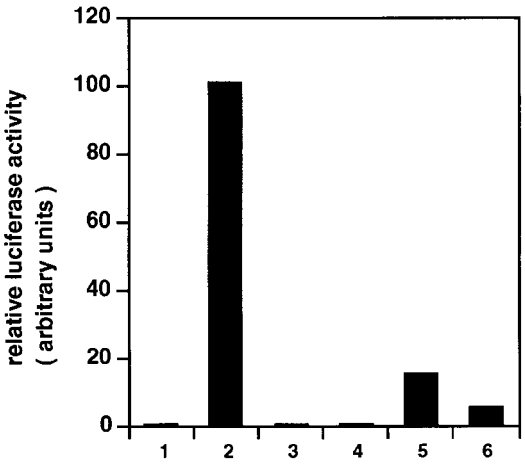
corticoid induction of gene transcription. Again this process is activation dependent and occurs only upon Stat5 phosphorylation and dimerization.

This principle of mutual functional regulation can be extended to other members of the steroid hormone receptor family. Although less pronounced, we observe that the mineralocorticoid receptor and the progesterone receptor are able to enhance the action of Stat5 with respect to transcription from the β-casein gene promoter. In both cases we also observe the negative interference of activated Stat5 with the induction of MMTV LTR transcription by the mineralocorticoid receptor and the progesterone receptor. Again, the effect is strongest for the glucocorticoid receptor and less pronounced for the other two.

In contrast to the glucocorticoid receptor, mineralocorticoid receptor and progesterone receptor no effect on Stat5 mediated transcription was observed by the activation of the androgen receptor. A functional interaction, however, was found again when the estrogen receptor and Stat5 were simultaneously activated. Estrogen receptor activation decreased the Stat5 mediated transcription of the β-casein luciferase indicator and Stat5 inhibited the estrogen receptor mediated transcription of a ERE-tk-luciferase construct.

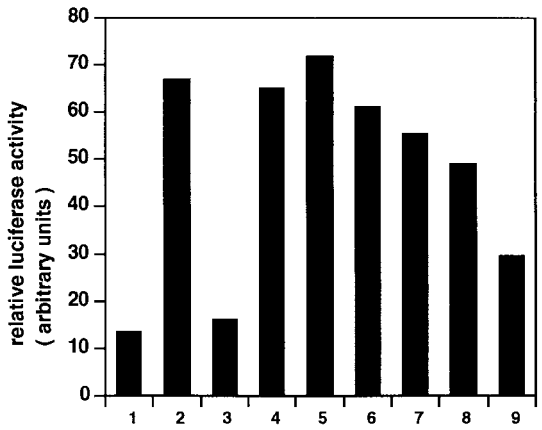
What kind of mechanisms can be envisaged for these functional interactions between Stat5 and the steroid hormone receptors? We have shown previously that Stat5 and the glucocorticoid receptor form a physical complex and we would assume that a similar situation might occur with the mineralocorticoid receptor and the progesterone receptor. However, no experiments have been done to show a direct interaction between those two transcription factors. A different situation might explain the results of the experiments in which Stat5 and the estrogen receptor were simultaneously activated. Both, Stat5 dependent transcription and estrogen receptor dependent transcription were inhibited. It is most likely that the activated transcription factors compete for a common interacting protein. p300/CBP or members of the p160 coactivator family are possible candidates. A similar situation might explain the modulation of AP-1 activity by the glucocorticoid receptor [24].

The interaction between the steroid hormone regulated transcriptional pathways and the cytokine regulated transcriptional pathways offers possibilities to



	1	2	3	4	5	6
β-casein-luc	+	+	+	+	+	+
MGF-STAT5	+	+	+	+	+	+
Prl R	+	+	+	+	+	+
GR	+	+	-	-	-	-
ER	-	-	+	+	+	+
DEX	-	+	-	-	-	-
PRL	-	+	-	-	+	+
ESTROGEN	-	-	-	+	-	+

Fig. 6. Stat5 induction of the β-casein gene promoter is repressed by estrogen receptor activation. COS7 cells were transiently cotransfected with constructs encoding Stat5 (MGF-Stat5), the prolactin receptor (Prl R), the glucocorticoid receptor (GR) (lanes 1 and 2) or the estrogen receptor (ER) (lanes 3 to 6), the β-casein-luciferase reporter gene (β-casein-luc) and an expression plasmid encoding β-galactosidase to monitor transfection efficiency. Luciferase activities were determined in extracts from cells induced with hormones indicated for 16 h and normalized to the β-galactosidase activities.



	1	2	3	4	5	6	7	8	9
ERE-tk-luc	+	+	+	+	+	+	+	+	+
MGF-STAT5	-	-	-	-	0.5	1	5	7.5	10
Prl R	-	-	+	+	+	+	+	+	+
ER	+	+	+	+	+	+	+	+	+
PRL	-	-	-	+	+	+	+	+	+
ESTROGEN	-	+	-	+	+	+	+	+	+

Fig. 7. Stat5 induction represses the estrogen receptor activation of an estrogen responsive gene promoter in a dose dependent manner. COS7 cells were transiently cotransfected with constructs encoding Stat5 (MGF-Stat5) (lanes 5 to 9), the prolactin receptor (Prl R) (lanes 3 to 9), the estrogen receptor (ER), the estrogen-luciferase reporter gene (ERE-tk-luc) and an expression plasmid encoding β-galactosidase to monitor transfection efficiency. Luciferase activities were determined in extracts from cells induced with hormones indicated for 16 h and normalized to the β-galactosidase activities.

explain tissue and cell type specificity of these extracellular signals. It is conceivable that regulatory mechanisms function at the level of expression of the components involved but also secondary modifications might be responsible for particular interactions [25,53–56]. The wide expression of Stat molecules and nuclear hormone receptors and their specific physiological functions in individual cell types might require such combinatorial regulation [57]. It will be interesting to see, if other members of the Stat family or the nuclear receptor family undergo similar functional modifications with ligand dependent transcription factors.

Acknowledgements

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