Functional interactions between Stat5 and the glucocorticoid receptor

Elisabeth Stöcklin, Manuela Wissler, Fabrice Gouilleux, Bernd Groner

Functional interactions between Stat5 and the glucocorticoid receptor

Elisabeth Stöcklin, Manuela Wissler, Fabrice Gouilleux & Bernd Groner

Institute for Experimental Cancer Research, Tumor Biology Center, Breisacher Strasse, 117, D-79106 Freiburg, Germany

SIGNAL transduction pathways enable extracellular signals to activate latent transcription factors in the cytoplasm of cells. Dimerization, nuclear localization and binding to specific DNA sequences result in the induction of gene transcription by these proteins. These events are necessary for the functioning of the JAK/STAT pathway and of the glucocorticoid-receptor pathway. In the former, the protein Stat5, which is a member of a family of signal transducers and activators of transcription, is activated by cytokines, hormones and growth factors[1–7]. These polypeptide ligands bind at the outside of the cell to specific transmembrane receptors and activate intracellular Janus protein tyrosine kinases (JAKs) to tyrosine-phosphorylate STAT proteins; interaction with the SH2 domain of the dimerization partner then confers the ability to bind to DNA at the STAT-response element and induce transcription[8–10]. In the glucocorticoid-receptor pathway, the receptor interacts with its steroid hormone ligand in the cytoplasm, undergoes an allosteric change that enables the hormone receptor complex to bind to specific DNA-response elements (glucocorticoid response elements, or GRE) and modulate transcription[11,12]. Although these pathways appear to be unrelated, we show here that the glucocorticoid receptor can act as a transcriptional co-activator for Stat5 and enhance Stat5-dependent transcription. Stat5 forms a complex with the glucocorticoid receptor which binds to DNA independently of the GRE. This complex formation between Stat5 and the glucocorticoid receptor diminishes the glucocorticoid response of a GRE-containing promoter.

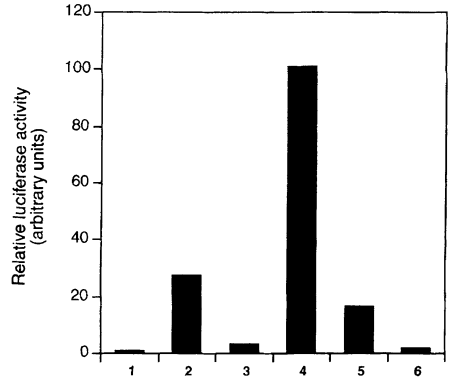
We used a COS cell co-transfection assay to study the hormonal synergism between prolactin and glucocorticoid hormones. COS cells do not express endogenous prolactin receptor or Stat5, a factor that confers the response to prolactin[13–15], and only trace amounts of glucocorticoid receptor are present[16]. We introduced a glucocorticoid receptor gene together with Stat5, the prolactin receptor[17], and a β-casein-gene-promoter luciferase construct into COS cells (Fig. 1). Induction of the cells with prolactin resulted in ~10-fold increase in luciferase activity over untreated cells (lanes 2 and 5), confirming previous results[15,18,19]. Glucocorticoid hormone treatment had either no or only a very minor effect (lane 3). Simultaneous induction with prolactin and glucocorticoid hormone increased the luciferase activity ~40-fold (lane 4). This experiment shows that the synergism between prolactin and glucocorticoid hormone action, well documented in mammary epithelial cells[20–22], can be reconstituted in transfected COS cells.

The β-casein promoter element used in the transfection experiments (Fig. 1) is either not or only very weakly affected by the activated glucorticoid receptor. It comprises binding sites for several nuclear factors[22] but does not contain a GRE consensus sequence[23]. To investigate the mechanism of synergism in transcriptional induction between Stat5 and the glucocorticoid receptor, we tested for complex formation in a co-immunoprecipitation experiment (Fig. 2). COS cells were transfected with Stat5, the prolactin receptor, and the glucocorticoid-receptor genes, and induced with prolactin and glucorticoid hormone. Nuclear extracts were prepared and immunoprecipitated with a Stat5-specific antiserum; immunoprecipitates were developed on western blots with a glucocorticoid-receptor-specific antiserum (Fig. 2*a*). The glucocorticoid receptor can be co-precipitated with Stat5 in cells induced by dexamethasone and prolactin (lane 4), indicating that a direct protein–protein interaction, independent of DNA binding, occurs between both transcription factors in the induced state. The result was consistent when the glucocorticoid-receptor-specific antiserum was used for immunoprecipitation and the Stat5-specific antiserum for western blotting (Fig. 2*b*).

Complex formation between Stat5 and the glucocorticoid receptor was confirmed in electrophoretic mobility shift essays (EMSA). A protein–DNA complex induced by treatment of COS cells transfected with mammary gland factor (MGF)–Stat5 and glucocorticoid-receptor genes and treated with prolactin and dexamethasone (Fig. 2*c*, lanes 10–12) could be supershifted with an antiserum specific for Stat5 (lane 11) or glucocorticoid receptor (lane 12). Nuclear extracts from cells transfected with MGF–Stat5 (lanes 1–4) or glucocorticoid receptor genes (lanes 5–8) served as controls.

The mouse mammary-tumour virus long terminal repeat (MMTV-LTR) contains a promoter region with several glucocorticoid-receptor binding sites (GRE) and responds to induction by glucocorticoid hormones[24,25]. Introduction of a MMTV-LTR luciferase gene and the glucocorticoid receptor into COS cells results in a strong inducibility by glucocorticoid hormone (Fig. 3, lane 2). Simultaneous introduction of Stat5 and activation of Stat5 by prolactin strongly suppresses the response of the MMTV-LTR towards glucocorticoids (Fig. 3, lane 3). This effect is not just due to the presence of Stat5, but requires Stat5 activation through the prolactin receptor (Fig. 3, lane 4).

The experiments described shed light on the synergistic action between Stat5 and the glucocorticoid receptor in the induction of the β-casein gene in mammary epithelial cells. Stat5 and glucocorticoid receptor form a molecular complex and cooperate in transcriptional induction. We believe that the strong transactivation domain of the glucocorticoid receptor enhances Stat5 action—the transactivation domain of Stat5 by itself is relatively weak[26]. The complex formation between Stat5 and the glucocorticoid receptor is an interesting example of cooperation between apparently unrelated signalling pathways. In the presence of glucocorticoid hormone, only the glucocorticoid receptor is activated to induce



β-Casein-luc	+	+	+	+	+	+
MGF-Stat5	+	+	+	+	+	+
Prl R	+	+	+	+	+	+
GR	+	+	+	+	-	-
Dex	-	-	+	+	-	-
Prl	-	+	-	+	+	-

FIG. 1 Stat5-mediated prolactin induction of the β-casein gene promoter is enhanced by glucocorticoid-receptor activation. COS7 cells were transfected with constructs encoding Stat5 (MGF–Stat5), the prolactin receptor (PrlR), the glucocorticoid receptor (GR) and a β-casein promoter for the luciferase gene (β-casein–luc). Luciferase activity was measured after treatment of the cells with no hormone (lane 1), prolactin (Prl, lane 2), dexamethasone (Dex, lane 3), dexamethasone and prolactin (lane 4). Cells in which the glucocorticoid receptor has been omitted from the transfection and which were treated with prolactin (lane 5) or no hormone (lane 6) were used as controls.

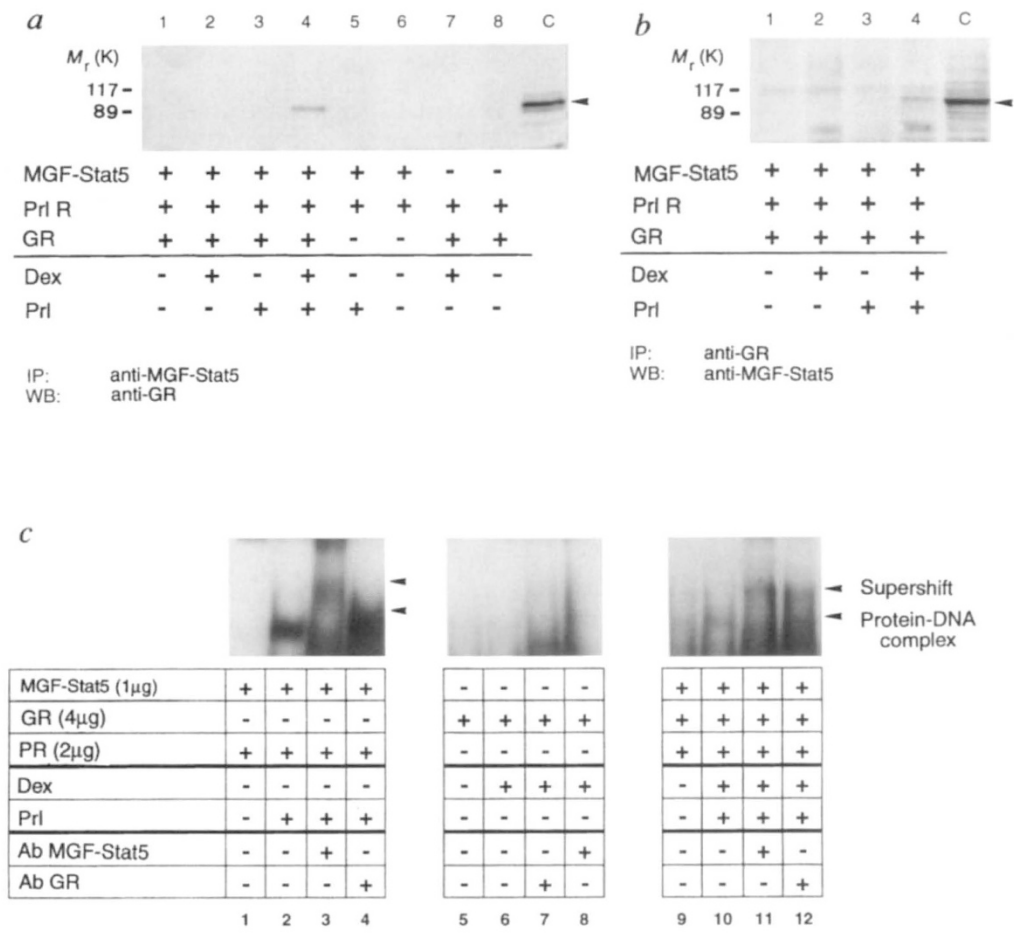


FIG. 2 Stat5 and glucocorticoid receptor from a molecular complex. *a* and *b*, COS7 cells were transfected with constructs encoding Stat5, glucorticoid receptor and prolactin receptor. Cells were induced with no hormones (lane 1), dexamethasone (lane 2), prolactin (lane 3) or dexamethasone and prolactin (lane 4). Nuclear extracts were prepared from the induced cells and immunoprecipitated (IP) with Stat5-specific antiserum (*a*) or with glucocorticoid-specific antiserum (*b*). Immunoprecipitates were separated by SDS–PAGE and western blotted (WB) with a glucocorticoid-specific antiserum (*a*) or with Stat5-specific antiserum (*b*). Cells in which either glucocorticoid receptor (lanes 5 and 6 in *a*) or Stat5 had been omitted from the transfections (lanes 7 and 8 in *a*) were used as controls. Extracts from COS cells transfected with glucocorticoid receptor (lane C in *a*) or Stat5 (lane C in *b*) served as a control for the western blot. *c*, Nuclear extracts were prepared from cells transfected with MGF–Stat5 (lanes 1–4), with glucocorticoid receptor (lanes 5–8), and with MGF–Stat5 and glucocorticoid receptor (lanes 9–12). Cells shown in lanes 2–4 were induced with prolactin, in lanes 6–8 with dexamethasone, and in lanes 10–12 with prolactin and dexamethasone. EMSA was done as described[3]. Stat5-specific antiserum was included in the DNA-binding reaction shown in lanes 3, 8 and 11; glucocorticoid-receptor-specific antiserum was included in the DNA-binding reaction shown in lanes 4, 7 and 12. PR, Prl R (prolactin receptor).

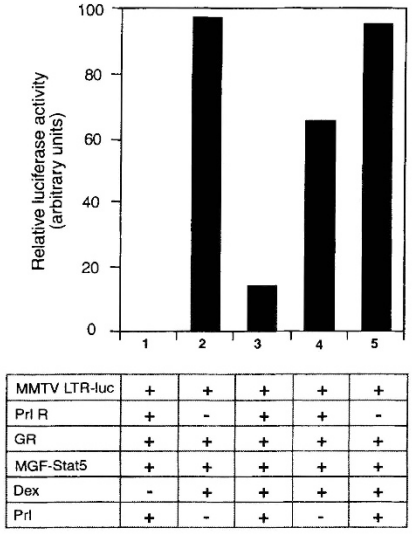


FIG. 3 Complex formation between Stat5 and the glucocorticoid receptor suppresses glucocorticoid induction of the MMTV-LTR promoter. COS 7 cells were transfected with constructs encoding a MMTV-LTR luciferase, the prolactin receptor, the glucocorticoid receptor and Stat5. Cells were induced with prolactin (lane 1), dexamethasone (lane 2), or dexamethasone and prolactin (lane 3). Cells in which the prolactin receptor has been omitted from the transfection were used as controls (lanes 2 and 5).

GRE-containing promoters; prolactin by itself weakly activates the Stat5-binding-site-containing promoters. In the presence of both signals, a molecular switch downregulates GRE-containing promoters and upregulates Stat5-response element-containing promoters. As cytokines can mediate inhibition of glucocorticoid-induced apoptosis of lymphocytes[27], it may be that Stat-mediated suppression of gene induction by the glucocorticoid receptor is responsible for this anti-apoptotic effect.

It has been proposed that the glucocorticoid receptor may act differently in its role as a negative transcriptional regulator in its interactions with AP-1 (ref. 28) and NF-κB (ref. 29). Our results define a new function for the glucocorticoid receptor as a ligand-dependent co-activator that is independent of the specific DNA-binding domain of the receptor. The synergism between Stat5 and glucocorticoid receptor survives in glucocorticoid-receptor variants lacking a GRE-binding function. The integrity of the Stat5-response element in the β-casein gene promoter and the activation of Stat5 through phosphorylation of a tyrosine residue at position 694 are prerequisites for the synergistic action of Stat5 and the glucocorticoid receptor (data not shown). There may be different co-activators for Stat5 that depend on individual cytokine signals and cell types and contribute to the signalling specificity[30]. Such a model could explain the versatility of Stat5 in different cytokine signalling pathways. □

Methods

Cell culture and transfection. Transfection experiments were done using the calcium phosphate precipitation technique. Half-confluent COS7 cells in 10-cm

dishes were transfected with 2 μg prolactin receptor expression vector, 2 μg glucocorticoid receptor expression vector, 2.5 μg Stat5 (pXM-MGF/Stat5). In experiments with reporter assays, 2 μg of the (−344/−1) β-casein gene promoter or 2 μg of the MMTV-LTR luciferase gene construct were used. In addition, 2 μg plasmid pCH110 encoding the β-galactosidase gene were included in each transfection as an internal control for transfection efficiency. Where indicated, the transfected cells were treated with 10^{-7} M dexamethasone and/or 5 μg ml^{-1} ovine prolactin for 16 h before collection for luciferase assays or for 1 h for complex formation assays.

Luciferase and β-galactosidase assays. Luciferase was assayed as described[18] in three independent experiments.

Preparation of nuclear extracts and electrophoretic mobility shift assays (EMSA). Subconfluent COS7 cells in 10-cm dishes were transfected and induced for 1 h before collection. Nuclear extracts were prepared[18]. Electrophoretic mobility shift assay and supershifts are described in ref. 3.

Co-immunoprecipitation assays. Nuclear extracts of co-transfected and prolactin- and dexamethasone-induced cells were prepared. The cleared supernatants were adjusted to 100 mM NaCl. Immunoprecipitations were carried out using polyclonal anti-Stat5a antibody[19] or glucocorticoid-receptor-specific antiserum[16] and immunoprecipitates isolated on protein A–Sepharose. Bound proteins were analysed by immunoblotting with polyclonal antiserum directed against either the glucocorticoid receptor or Stat5.

Received 17 June; accepted 9 September 1996.

1. Azam, M. *et al. EMBO J.* **14,** 1402–1411 (1995).
2. Fuji, H. *et al. Proc. Natl Acad. Sci. USA* **92,** 5482–5486 (1995).
3. Gouilleux, F. *et al. Endocrinol.* **136,** 5700–5708 (1995).
4. Gouilleux, F. *et al. EMBO J.* **14,** 2005–2013 (1995).
5. Mui, A., Wakao, H., O'Farrell, A. M., Harada, N. & Miyajima, A. *EMBO J.* **14,** 1166–1175 (1995).
6. Pallard, C. *et al. EMBO J.* **14,** 2847–2856 (1995).
7. Yoshimura, A. *et al. EMBO J.* **15,** 1055–1063 (1996).
8. Darnell, J. E., Kerr, I. M. & Stark, G. R. *Science* **264,** 1415–1421 (1994).
9. Ihle, J. N. *Nature* **377,** 591–594 (1995).
10. Ihle, J. N. *Cell* **84,** 331–334 (1996).
11. Beato, M., Herrlich, P. & Schütz, G. *Cell* **83,** 851–857 (1995).
12. Mangelsdorf, D. J. *et al. Cell* **83,** 835–839 (1995).
13. Schmitt-Ney, M., Doppler, W., Ball, R. K. & Groner, B. *Mol. Cell. Biol.* **11,** 3745–3755 (1991).
14. Schmitt-Ney, M., Happ, B., Ball, R. & Groner, B. *Proc. Natl Acad. Sci. USA* **89,** 3130–3134 (1992).
15. Wakao, H., Gouilleux, F. & Groner, B. *EMBO J.* **13,** 2182–2191 (1994).
16. Höck, W. & Groner, B. *J. Biol. Chem.* **265,** 5403–5408 (1990).
17. Lebrun, J.-J., Ali, S., Goffin, V., Ullrich, A. & Kelly, P. *Proc. Natl Acad. Sci. USA* **92,** 4031–4035 (1995).
18. Gouilleux, F., Wakao, H., Mundt, M. & Groner, B. *EMBO J.* **13,** 4361–4369 (1994).
19. Liu, X., Robinson, G. W., Gouilleux, F., Groner, B. & Hennighausen, L. *Proc. Natl Acad. Sci. USA* **92,** 8831–8835 (1995).
20. Doppler, W., Groner, B. & Ball, R. K. *Proc. Natl Acad. Sci. USA* **86,** 104–108 (1989).
21. Groner, B., Altiok, S. & Meier, V. *Mol. Cell. Endocrinol.* **100,** 109–114 (1994).
22. Groner, B. & Gouilleux, F. *Curr. Op. Gen. Develop.* **5,** 587–594 (1995).
23. Welte, T., Phillipp, S., Cairns, C., Gustafsson, J.-A. & Doppler, W. *J. Steroid Biochem. Mol. Biol.* **47,** 75–81 (1993).
24. Hynes, N. E., Kennedy, N., Rahmsdorf, U. & Groner, B. *Proc. Natl Acad. Sci. USA* **78,** 2038–2042 (1981).
25. Hynes, N. E. *et al. Proc. Natl Acad. Sci. USA* **80,** 3637–3641 (1983).
26. Moriggl, R. *et al. Mol. Cell. Biol.* **16,** 5691–5700 (1996).
27. LaVoie, H. A. & Witorsch, R. J. *Proc. Natl Soc. Exp. Biol. Med.* **209,** 257–269 (1995).
28. Teurich, S. & Angel, P. *Chem. Senses* **20,** 251–255 (1995).
29. Cato, A. C. B. & Wade, E. *BioEssays* **18,** 371–378 (1996).
30. Ivashkiv, L. B. *Immunity* **3,** 1–4 (1995).

ACKNOWLEDGEMENTS. We thank S. Berchtold, E. Pfitzner, R. Moriggl, C. Beisenherz and M. Fritsche for valuable discussions and I. Fernandez for editorial assistance.

CORRESPONDENCE and requests for materials should be addressed to B.G.

An oxysterol signalling pathway mediated by the nuclear receptor LXRα

Bethany A. Janowski*, Patricia J. Willy*, Thota Rama Devi*, J. R. Falck† & David J. Mangelsdorf*

* Howard Hughes Medical Institute and Department of Pharmacology,
† Department of Molecular Genetics, University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Blvd, Dallas, Texas 75235-9050, USA

CHOLESTEROL and its oxysterol congeners are important constituents of cell membranes and function as intermediates in several crucial biosynthetic pathways. These compounds autoregulate their metabolic fate by end-product repression and activation of downstream catabolism[1]. Although end-product repression by oxysterols is relatively well understood[2], the mechanism by which these compounds act as positive transcription signalling molecules is unknown. Here we identify a specific group of endogenous oxysterols that activate transcription through the nuclear receptor LXRα. Transactivation of LXRα by oxysterols occurs at concentrations at which these compounds exist *in vivo*. The most potent activators also serve as intermediary substrates in the rate-limiting steps of three important metabolic pathways: steroid hormone biosynthesis, bile acid synthesis, and conversion of lanosterol to cholesterol. Our results demonstrate the existence of a nuclear receptor signalling pathway for oxysterols and suggest that LXRα may be important as a sensor of cholesterol metabolites.

Human LXRα is an orphan member of the nuclear receptor superfamily, which has the potential to function as a ligand-dependent transcription factor when complexed with its heterodimeric partner, the retinoid-X receptor (RXR)[3]. To identify LXRα ligands, we prepared concentrated lipid extracts from a variety of tissues and tested for ability to activate LXRα in a high-throughput cotransfection assay similar to that used to identify ligands for other receptors[4,5]. For the initial screening, a chimaeric receptor was used in which the ligand-binding domain of LXRα was fused to the DNA-binding domain of the yeast transcription factor GAL4 (ref. 3). The resultant GAL4-LXRα expression plasmid was cotransfected together with a GAL4-responsive luciferase reporter plasmid into CV-1 cells and challenged with concentrates from several tissue sources. A significant (six-fold) induction of luciferase activity was seen with lipid extracts derived from breeding-bull testis (Fig. 1*a*). The migration of this lipid activity on reverse-phase high-pressure liquid chromatography (HPLC) (data not shown) indicates that the compound may be related to a class of meiosis-activating sterols (MAS) isolated from gonads[6]. To explore the possibility that these sterols were LXRα

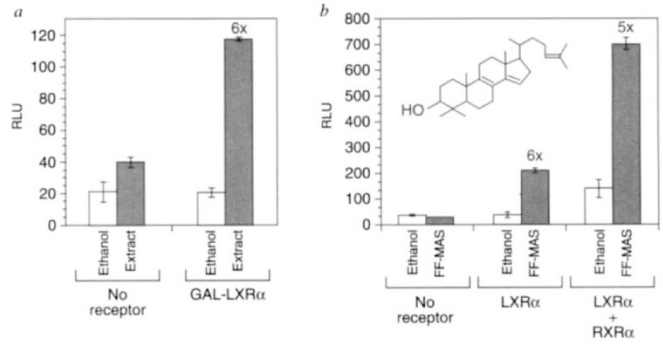


FIG. 1 Human LXRα is activated by gonad-specific sterols. *a*, Transactivation of LXRα ligand-binding domain (LBD) with testis extract. CV-1 cells were cotransfected with a GAL4-responsive luciferase reporter plasmid and an expression plasmid encoding no receptor or a chimaeric receptor composed of the GAL4 DNA-binding domain fused to the LXRα LBD (GAL4–LXRα)[3]. After transfection, cells were treated with ethanol or 1% of the concentrated testis extract. *b*, Transactivation of LXRα with follicular-fluid meiosis-activating sterol (FF-MAS). CV-1 cells were cotransfected with an LXRα-responsive reporter plasmid and the receptor expression plasmids for LXRα alone or with RXRα. After transfection, cells were treated with ethanol or 50 μM FF-MAS. Inset, structure of FF-MAS. RLU, Relative light units.