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Communication

A role for the non-conserved N-terminal domain of the TATA-binding protein in the crosstalk between cell signaling pathways and steroid receptors

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Abstract: Transcriptional induction by steroid receptors is coupled to cellular signal transduction pathways although, in general, the mechanisms governing these events are not well defined. Using TATA-binding protein (TBP) specificity mutants that recognize a TGTA box, we show that yeast TBP expressed in mammalian cells can support steroid-mediated gene induction to a similar degree as human TBP, however yeast TBP does not support the 8-Bromo-cAMP-mediated potentiation of glucocorticoid receptor (GR)-dependent transactivation. Chimeras between yeast and human TBP reveal that it is the non-conserved N-terminus of TBP that governs the potentiation of GR action. While the conserved core of TBP is sufficient for TATA-element binding and preinitiation complex formation, the role of the N-terminus has remained elusive. Our results suggest a role of the N-terminus of human TBP in coupling cell signaling events to steroid-mediated transcription, thereby establishing one of the few described functional roles of this polypeptide domain in a physiological process.

Keywords: TBP; glucocorticoid receptor; cyclic AMP; steroid receptor; coactivator; crosstalk; protein kinase A

1. Introduction

The 180 amino acid core of the TATA-binding protein (TBP) is conserved across virtually all eukaryotes. This core domain is sufficient for TATA-element binding and preinitiation complex formation. TBP also contains an N-terminal region that is highly divergent across species and whose function remains incompletely understood. Although TATA element recognition by TBP is

necessary for the assembly of the basal transcription apparatus at a promoter, it is not sufficient for regulated transcription by upstream factors. This activity is dependent on the TBP-associated factors (TAFs) which, together with TBP, comprise the large TFIID protein complex [1]. TFIID functions in the first step of transcription initiation by binding to the TATA element and allowing the subsequent incorporation of the other transcription initiation factors including TFIIB, TFIIE, TFIIF, TFIIH and RNA polymerase II [2].

Steroid hormone receptors belong to a superfamily of nuclear receptors that can function as ligand-dependent transcription factors [3]. Communication by nuclear receptors with the core transcription apparatus is often thought to be indirect, through intermediaries or adaptors termed coactivators or corepressors. A wide variety of these coregulators have been identified by their ability to physically interact with nuclear receptors, particularly Activation Function 2 (AF2), an evolutionarily conserved hormone-dependent activation domain present in the C-terminus of nuclear receptors [4]. The mode of interaction of coregulators with the basal transcription apparatus is generally not known, but several enzymatic activities have been ascribed to coregulators including histone methyltransferase, acetyltransferase, deacetylase, and ubiquitin ligase [5,6,7]. However, multiple members of the steroid receptor family and the nuclear receptor superfamily have been reported to interact directly with identified TAFs and in some cases directly with TBP itself. The glucocorticoid receptor [8], progesterone receptor [9], and estrogen receptor alpha [10] interact directly with TBP *in vitro* where TBP imposes structure on the inherently unstructured N-terminal domains of the respective receptors associated with Activation Function 1 (AF1) of the receptors.

A novel approach to the study of mechanisms of transcriptional activation by nuclear receptors was taken by Stunnenberg and co-workers. They have shown that transactivation by the retinoic acid receptor in an embryonal carcinoma cell line is dependent on an activity analogous to the adenovirus E1A protein. They demonstrated functional cooperation between this “E1A-like” activity and human TBP. In contrast, yeast TBP was unable to cooperate with this coactivator to support retinoic acid receptor-mediated gene induction [11]. The domain of human TBP that promotes this function was mapped to the conserved C1a domain by using yeast-human TBP chimeras [11]. Similarly, yeast TBP is unable to support Sp1 mediated activation of transcription [12]. These data suggest an interaction of coactivators with transcription factors and TBP. These studies were facilitated by using TBP mutants with altered TATA box-binding specificity [13]. The use of these TBP specificity mutants (TBPspms) permits the examination of transcription of reporter genes containing a TGTA box with little interference from the endogenous TBP.

Steroid receptor signaling can be modulated by activation of many cellular signaling pathways. The coupling of steroid receptor signaling to other signal transduction pathways can be manifested in several ways, extra-nuclear or so called “non-genomic” actions of steroid receptors, ligand-independent activation, and the modulation of ligand-dependent genomic actions. As an example of the later we have shown that activation of a variety of signal transduction pathways can potentiate hormonal induction of promoters targeted by the glucocorticoid and progesterone receptors [14-19]. These manipulations include activation of protein kinases A and C, inhibition of protein phosphatases, activation of epidermal growth factor receptors, and cell stress. Since then, modulation of GR signaling by many signaling pathways in numerous cell types has been reported. In general, the mechanisms that couple steroid receptors with other signaling pathways are poorly defined but the focus has been on phosphorylation of receptors or coregulators [20,21,22].

The work from the Stunnenberg laboratory investigating promoter and cell-type specific actions

of the retinoic acid receptor [11] prompted us to test whether TBP might also play an active role in crosstalk of steroid receptors and intracellular signal transduction pathways. In the present work, yeast/human TBP complementation assays like those described above were used to identify the domains of TBP necessary for coupling signal transduction pathways and hormone-dependent transcription mechanisms. Human and yeast TBP specificity mutants were analyzed for their ability to support steroid hormone-dependent transcriptional potentiation by 8-Bromo cAMP, an activator of cAMP-dependent protein kinases. These studies identify a region within the non-conserved N-terminus of human TBP that supports potentiation of the steroid response by activation of cell signaling pathways. This is one of the first examples where the N-terminal domain of TBP can be linked to a physiological process.

2. Materials and Methods

2.1. Cell culture and transfection

The Ltk- mouse fibroblast cell line [23] was propagated and transfected as previously described [16] using plasmids at 2 μg/mL in the DMSO/DEAE-dextran transfection solution. Sixteen hours after transfection the cells were treated with dexamethasone (10nM) or vehicle (ethanol) with 8-Br-cAMP (1mM) or vehicle (H_2O) for 24 hours. Luciferase activity was determined as described previously [16]. Duplicate culture dishes were assayed and the results normalized to the protein content of the cell extracts. All transfection experiments were performed 5–7 times. U937 cells [24] were maintained in RPMI medium.

2.2. Plasmids

Human and yeast TBPspm mammalian expression plasmids (pSG5hTBPspm and pSG5yTBPspm) were gifts from Dr. H. Stunnenberg and have been previously described [11]. In order to make chimeric TBPspms, a unique MroI site was introduced at the border of the N-terminal domain and the conserved core of both human and yeast TBPspm by oligo-directed mutagenesis (U.S.E. mutagenesis kit, Pharmacia). MroI-BamHI fragments were then exchanged to generate the pSG5hyTBPspm and pSG5yhTBPspm TBP fusion expression plasmids. Direct-rapid mutagenesis [25] was used to create an in-frame deletion of the polyglutamine motif of the human TBP N-terminal domain. Following PCR amplification from the template pSG5hTBPspm using the primers 5'-ATGC**CTCGAG**TTCCAAAATAGACAG-3' and 5'-ATGC**CTCGAG**GCAGTGGCAGCTGCA-3', the products were digested with XhoI to create cohesive termini, gel purified and ligated yielding the expression plasmid h(ΔQ)TBPspm.

To assess N-terminal TBP sequences as kinase substrates the yeast and human domains were made as GST fusion proteins. To construct these vectors the coding sequences of human (residues 1–159) and yeast (residues 1–61) TBP N-termini were amplified and cloned into pGEX (Pharmacia) GST expression plasmids pGEX4T3 (human) and pGEX4T1 (yeast). The fusion proteins were expressed in *E. coli* strain BL21 and purified over glutathione resin according to the manufacturer's protocol. The human and yeast fusion proteins were designated GST-HN and GST-YN respectively. DNA sequencing was performed to confirm reading frame of all expression constructs.

A luciferase reporter plasmid with an altered TATA box was made by inserting the

oligonucleotide (5'-GCT**TGTA**AAGGT-3' and its complement into the filled-in XhoI site of the promoterless luciferase vector, pXP2 [26]. Into this vector, pTGTAluc, an oligonucleotide with an optimized hormone response element [27] 5'-TCGAGGAACAGTTTGTACCG-3' was inserted into the SalI site of pTGTAluc. Clones were recovered that contained two GRE sequences in tandem and designated p2HRETGTAluc.

2.3. In vitro kinase assay

10 μg of whole cell lysate from U937 and Ltk- mouse fibroblast cells (PMA, 8-Br cAMP or vehicle treated) was incubated with approximately 1 μg of GST, GST-HN or GST-YN bound to glutathione beads for 15 min at 30 °C in a total volume of 50 μL. The kinase incubation buffer contained 20 μM ATP, 50 mM Tris-HCl (pH 7.6), 1 mM $MgCl_2$, 5 mM NaF, 1 mM vanadate and 0.1 μCi/uL ^{32}P-γATP. After incubation, the beads were washed three times with PBS and samples analyzed by SDS-PAGE followed by autoradiography.

3. Results

3.1. Differential ability of human and yeast TBP to support potentiation of GR-mediated transcription by 8-Bromo cAMP

Activation of the cAMP pathway in Ltk- fibroblasts with forskolin, an activator of protein kinase A, or by treatment with 8-Bromo cAMP potentiates the glucocorticoid-dependent induction of a target promoter [17]. To investigate a possible role for TBP and TBP associated proteins in steroid-mediated transcription and its potentiation by 8-Bromo cAMP, we used a reporter gene with a mutant TATA box that is dependent on introduced TBP specificity mutants (TBPspms) that recognize the sequence TGTA. Plasmids for human TBP specificity mutants (pSG5hTBPspm) or yeast TBP specificity mutants (pSG5yTBPspm) were cotransfected into mouse Ltk- fibroblasts along with a hormone-responsive reporter plasmid with an altered TATA (TGTA) box. All transfection experiments represent data from 5 to 7 independent experiments. As shown in Figure 1A, expression of either human or yeast TBPspm resulted in induction of luciferase activity following dexamethasone treatment. This indicates that both hTBPspm and yTBPspm support GR-mediated gene transcription. Furthermore, when cells were treated with a combination of dexamethasone and 8-Bromo cAMP, we observed a very strong potentiation of luciferase activity (more than 7 fold over dexamethasone alone) for hTBPspm transfectants indicating that hTBPspm is fully capable of supporting 8-Bromo cAMP-mediated potentiation of GR-directed gene transcription. In contrast, potentiation by 8-Bromo cAMP was considerably compromised when yTBPspm directed transcription. The average fold potentiation (ratio of luciferase activity with dexamethasone + 8-Bromo cAMP over dexamethasone alone) from 7 experiments averaged 7.4 ± 0.3 s.e. for the human TBP and 2.3 ± 0.3 for yeast (Figure 1B). In the absence of exogenous TBPspm, minimal induction of luciferase activity was seen, suggesting that transcription of the reporter is largely mediated by the transfected TBPspm (Figure 1).

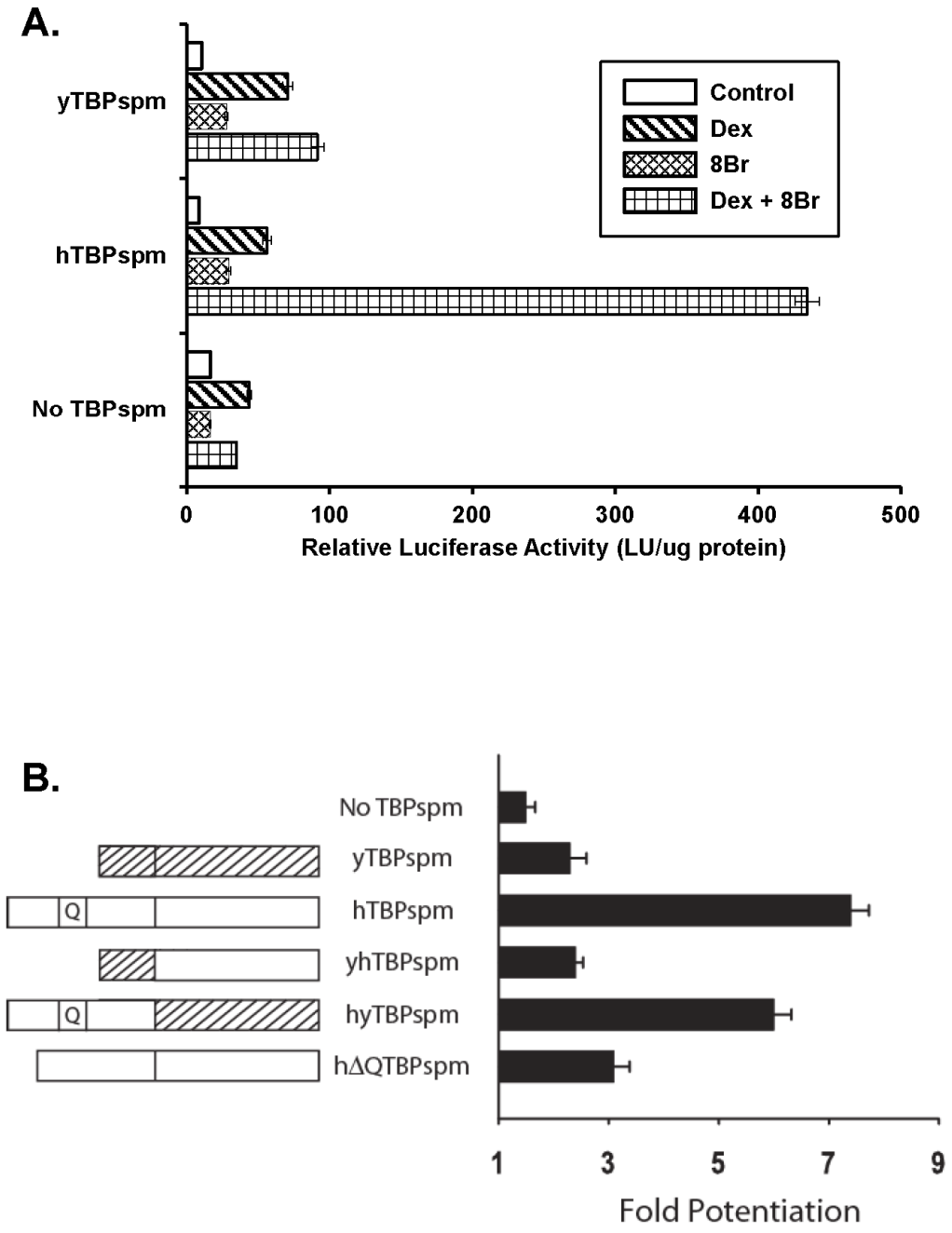

Figure 1. Involvement of the N-terminus of human TBP in potentiation of GR-mediated transcription by 8-Br-cAMP. (A) Glucocorticoid-mediated induction and its potentiation by 8-Br-cAMP directed by yeast and human TBPspms from the GR-inducible reporter, p2HRETGTALuc. Dex: dexamethasone, 8Br: 8-Br cAMP. Data is from a representative experiment. Bars denote the range of inductions seen in duplicate pairs of transfections. (B) Schematic representation of TBPspms and the potentiation of dexamethasone induction mediated by 8-Br cAMP. Fold potentiation is defined as (Dex + 8-Br cAMP)/(Dex) luciferase activities. Data represents an average derived from 5–7 independent experiments. Errors bars depict the standard error of the mean.

3.2. The N-terminus of human TBP mediates potentiation of GR-mediated gene transcription by 8-Bromo cAMP

In order to determine the residues of human TBP that support potentiation of GR-mediated gene transcription by 8-Bromo cAMP, we constructed expression plasmids for human-yeast TBPspm chimeras. Because the C-terminal core domain of human and yeast TBP have 80% amino acid identity, we initially focused on the non-conserved N-terminal domain. The human N-terminus (residues 1–159) was fused to the yeast C-terminal core domain to create the chimera, hyTBPspm, and the corresponding region of yeast TBP (residues 1–61) was fused to the human C-terminal core domain creating yhTBPspm. The chimeric TBPspms, depicted in Figure 1B, were transfected into Ltk- cells and their ability to support potentiation of hormone induction was compared to hTBPspm and yTBPspm. Potentiation tracked with the N-terminal domain of the TBPspm. Replacement of the human N-terminal domain with that of yeast reduced potentiation to that seen with yTBPspm. Thus yhTBP supported only minimal potentiation like yTBP. In contrast, hyTBPspm, in which N-terminal domain sequences of yTBPspm have been replaced with the human N-terminal domain, supported potentiation by 8-Bromo cAMP almost to the same level as full length human TBPspm (6.0 versus 7.4 fold, Figure 1B).

3.3. Deletion of the polyglutamine motif in the N-terminus of human TBP abrogates cAMP-dependent potentiation of GR action

The N-terminus of human TBP contains a 38 residue polyglutamine repeat not present in yeast TBP. Because Q tracts are thought to facilitate protein-protein interactions, we investigated whether this region is involved in GR-mediated potentiation of gene transcription by 8-Bromo cAMP. A human TBPspm expression plasmid was constructed that lacks the Q tract in the N-terminus of human TBPspm, h(ΔQ)TBPspm, and tested its ability to support potentiation by 8-Bromo cAMP. Deletion of the glutamine repeat reduced potentiation from 7.4 to 3.1 fold, suggesting that this region may serve as an interaction domain for a cofactor involved in coupling cell signaling pathways to steroid-mediated transcription (Figure 1B).

3.4. The N-terminus of human TBP is not phosphorylated in cells treated with 8-Bromo cAMP

Activation of the ERK kinase pathway in U937 leukemic cells induces phosphorylation of the N-terminus of human TBP [28]. Thus a mechanism whereby activation of protein kinase A by 8-Bromo cAMP results in phosphorylation of the N-terminus of human TBP as a signal for the recruitment of a cofactor necessary for potentiation of steroid induction could be envisioned. We performed *in vitro* kinase assays using whole cell extracts from Ltk- cells or from 8-Bromo cAMP-treated cells and purified GST fusion proteins with either the N-terminus of human (GST-HN) or yeast (GST-YN) TBP. As a positive control, cell extracts from U937 cells after activation of the ERK kinase pathway with PMA were used.

GST-HN was phosphorylated when incubated with PMA-activated U937 extracts but not in unactivated extracts (Figure 2). However, 8-Bromo cAMP treatment of U937 cells did not generate an extract that phosphorylated GST-HN. No phosphorylation of either GST-HN or GST-YN was observed using either PMA- or 8-Bromo cAMP-activated extracts of Ltk- cells. We conclude that

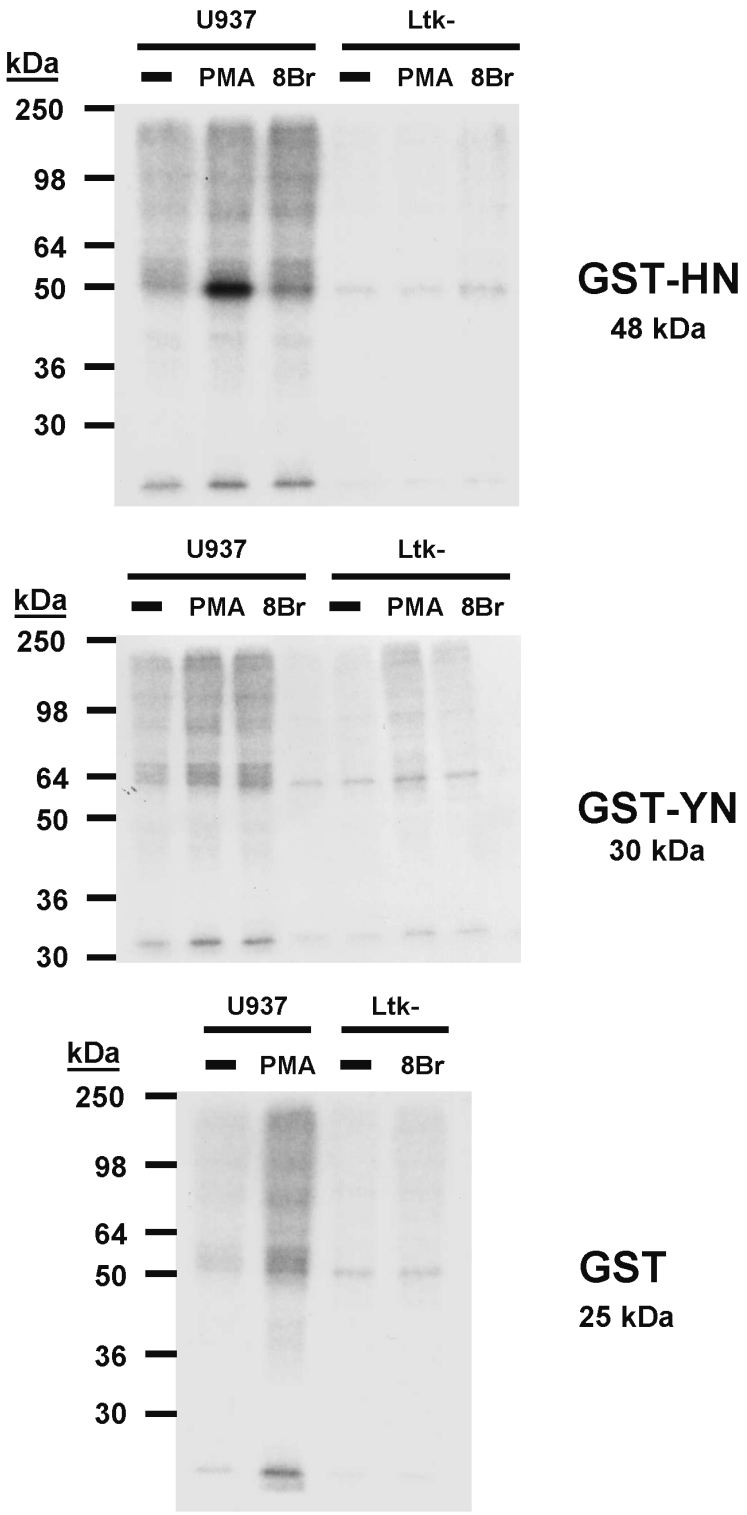

Figure 2. The N-terminus of human TBP is not phosphorylated in cell extracts from 8-Br-cAMP treated Ltk- fibroblasts. The kinase activity of control, PMA, and 8-Br cAMP-treated cell extracts from U937 leukemic cells and Ltk- fibroblasts was assessed by incubating with GST, GST-YN or GST-HN bound to glutathione beads and γ^{32}P ATP [19]. Phosphorylation of the GST substrates was visualized by autoradiography following SDS-PAGE.

8-Bromo cAMP-mediated potentiation of steroid induction is unlikely to involve phosphorylation of the N-terminal domain of human TBP. However, additional investigations are required to ascertain whether rapid, nongenomic signaling via hormone activated glucocorticoid receptors could result in the phosphorylation of the N-terminus of TBP. We also cannot rule out that dexamethasone induces expression of a kinase although we see full potentiation in as little as 4 hours of treatment, the soonest reliable measurements of luciferase activity can be made.

4. Discussion

The nuclear receptors comprise one of the largest families of transcription factors encoded by the mammalian genome and play diverse and critical roles in organismal physiology. The potential importance of the coupling of steroid receptors with other cellular signaling pathways is highlighted by observations that activation of certain pathways elicits agonist activity in clinically used steroid antagonists like tamoxifen [29] and RU486 [15,16], promotes glucocorticoid resistance in bronchial epithelial cells [30], and inhibits glucocorticoid-mediated apoptosis in T cell acute lymphoblastic leukemia [31]. These findings lead to a reshaping of our view of the steroid receptor superfamily as integrators of multiple signal transduction pathways rather than as mediators of a single signal.

Although much work has gone into elucidating the molecular mechanism of the crosstalk between cell signaling pathways and steroid hormone action, little is conclusively understood. GR contains at least five (human) to eight (mouse) phosphorylation sites and phosphorylation has been shown to impact DNA binding affinity, the interaction with coregulators or the transcription initiation complex, the intracellular movement of receptors as well as transcriptional output in a cell type and promoter specific fashion [32-35]. Activation of PKA in a rat hepatoma cell line promoted enhanced GR binding to the glucocorticoid responsive region of the rat tyrosine aminotransferase gene [36]. However, we were unable to see a change in the phosphorylation of GR in T47D mammary carcinoma cells following activation of protein kinase A with 8-Bromo cAMP or forskolin [17]. Phosphorylation of coregulators also has been implicated in the coupling of signaling pathways with steroid receptor dependent transcription. 8-Bromo cAMP has been shown to induce phosphorylation of steroid receptor coactivator 1 (SRC-1), a member of a family of coactivators that regulates steroid hormone action [37]. This, in turn, has been shown to facilitate ligand-independent activation of the chicken progesterone receptor and augment interactions with CBP [37]. Regulation of receptor-coregulator action by phosphorylation remains a promising area of investigation [21,38].

The means by which receptors communicate with the core transcription apparatus to increase transcription initiation is the subject of intense investigation. Studies have identified a large number of receptor-interacting proteins that are putative intermediaries or co-regulators of receptor action. We have used an approach working back from the core transcription apparatus employing using TBP specificity mutants to explore GR action and signal crosstalk. In mouse fibroblasts, yeast TBP fully supported GR-mediated induction of a minimal promoter bearing a TATA-box and two copies of an optimized hormone response element. However, the yeast TBP did not support crosstalk of signal transduction pathways with glucocorticoid receptor transactivation. Human TBP supported a 7.4-fold potentiation of hormone-induced transcription whereas with yeast TBP we see only a 2.3 fold potentiation (Figure 1B). Surprisingly, the ability to support 8-bromo cAMP-mediated potentiation mapped to the non-conserved N-terminal domain of TBP. A yeast core domain fused to a human

N-terminus fully supported 8-bromo cAMP potentiation, whereas a human core with a yeast N-terminus gave results equivalent to full length yeast TBP.

The involvement of the N-terminal domain of TBP in potentiation of glucocorticoid induction led us to ask whether its phosphorylation was induced by 8-bromo cAMP which in turn might promote the recruitment of cofactors of receptor-mediated transactivation. Biggs et al have shown that activation of the mitogen-activated protein kinase pathway by phorbol esters in U937 leukemic cells induces phosphorylation of the N-terminus of TBP *in vitro* [28]. Previously we had shown that phorbol ester treatment of the glucocorticoid responsive mammary carcinoma cell line, T47D(A1-2), also resulted in enhancement of glucocorticoid-mediated transcription [19]. Although we confirmed the phosphorylation of the N-terminus of TBP by extracts of PMA-activated U937 cells, we were not able to detect appreciable phosphorylation of the N-terminus of TBP following 8-Bromo cAMP treatment of mouse fibroblasts or U937 cells. Nonetheless, we cannot rule out that cAMP-induced phosphorylation of the N-terminal domain might occur in the context of the entire TBP molecule *in vivo*.

Another possible link of the N-terminal domain and steroid-mediated transactivation is found in studies indicating that the high mobility group protein, HMG-1, interacts with the N-terminus of TBP and that this interaction is mediated by the polyglutamine region which is important for stable HMG-1/TBP/TATA complex formation [39]. In these studies, they showed that HMG-1 increases the affinity of TBP for the TATA element 20-fold [39]. HMG-1 has also been shown to be a cofactor important for steroid hormone receptor transcriptional activity [40,41]. However, the fact that yTBPspm fully supports hormone response indicates that the poly glutamine region is not necessary for all transcriptional responses to hormone. Our own attempts to identify factors that interact with the human TBP N-terminus by yeast two-hybrid assays were thwarted by the intrinsic transcriptional activity of the human N-terminal domain in this assay (data not shown).

That the nonconserved N-terminus may mediate crosstalk with steroid receptors is surprising in that the 180 amino acid conserved core domain of TBP is sufficient for most of the analyzed functions of TBP including TATA-element binding, preinitiation complex formation, TAF association, and transcription initiation [42]. In contrast much less is understood about the function of the N-terminal region of mammalian TBP. A number of functions for the non-conserved N-terminal domain have been suggested, including roles in transcribing genes encoding RNAs involved in splicing, transcription of U6 snRNA, interactions with high mobility-group protein 1, DNA binding and cell proliferation [39,43-46]. However, studies on primary fibroblasts derived from mouse embryos homozygous for an allele of TBP lacking most of the N-terminus indicate that fundamental processes including proliferation, transcription initiation site fidelity, splicing and global gene expression were unaffected [47]. Nonetheless, the ΔN allele is an embryonic lethal in homozygotes and there is an effect on the maternal immunotolerance of pregnancy [48] indicating that, although the N-terminal domain may not play a global role, it does have functions critical for the organism. Our data is consistent with these findings suggesting that the N-terminus of TBP is dispensable for steroid-mediated transactivation but does play a more restricted role in the crosstalk of steroid receptors and cellular signaling pathways. Clearly, elucidation of the cellular pathways in which the N-terminal region of TBP participates is necessary for a mechanistic understanding of the numerous functions of TBP in eukaryotic transcription. This work ascribes a specific role for the N-terminus of TBP, a domain whose functional importance has remained elusive.

5. Conclusions

In summary, the present studies demonstrate a role of the N-terminus of human TBP in coordinating the communication between cyclic AMP signaling and GR transcriptional activity. Previous studies have demonstrated that activation of a variety of cellular signaling pathways can lead to potentiation of GR-mediated transcription. By exploiting specificity mutants of yeast and human TBP we show that human TBP, but not yeast TBP, supports robust potentiation of GR action by cyclic AMP. The use of chimeric TBPs demonstrated that it is the non-conserved N-terminal domain of human TBP that permits the coupling of cyclic AMP signaling and GR-mediated transcription. This work establishes a functional role for the poorly understood N-terminal domain of TBP and reshapes our view of steroid receptors as integrators of multiple signaling pathways.

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Conflict of interest

All authors declare no conflicts of interest in this paper.

References

1. Goodrich JA, Tjian R (1994) TBP-TAF complexes: selectivity factors for eukaryotic transcription. *Curr Opin Cell Biol* 6: 403-409.
2. Buratowski S (1994) The basics of basal transcription by RNA polymerase II. *Cell* 77: 1-3.
3. Evans RM (1998) The steroid and thyroid hormone receptor superfamily. *Science* 240: 889-895.
4. Dasgupta AB, Lonard DM, O'Malley BW (2014) Nuclear receptor coactivators: master regulators of human health and disease. *Annu Rev Med* 65: 279-292.
5. Xu J, Li Q (2003) Review of the *in vivo* Functions of the p160 Steroid Receptor Coactivator Family. *Mol Endocrinol* 17:1681-1692.
6. Smith CL, DeVera DG, Lamb DJ, et al. (2002) Genetic ablation of the steroid receptor coactivator-ubiquitin ligase, E6-AP, results in tissue-selective steroid hormone resistance and defects in reproduction. *Mol Cell Biol* 22: 525-535.
7. Beitel LK, Elhaji YA, Lumbroso R, et al. (2002) Cloning and characterization of an androgen receptor N-terminal-interacting protein with ubiquitin-protein ligase activity. *J Mol Endocrinol* 29: 41-60.
8. Khan SH, Ling J, Kumar R (2011) TBP binding-induced folding of the glucocorticoid receptor AF1 domain facilitates its interaction with steroid receptor coactivator-1. *PLoS One* 6: e21939
9. Kumar R, Moure CM, Khan SH, et al. (2013) Regulation of the structurally dynamic N-terminal domain of progesterone receptor by protein-induced folding. *J Biol* 288: 30285-3099.
10. Warnmark A, Wikstrom A, Wright AP, et al. (2001) The N-terminal regions of estrogen receptor alpha and beta are unstructured *in vitro* and show different TBP binding properties. *J Biol Chem* 276: 45939-45944.

11. Keaveney M, Berkenstam A, Feigenbutz M, et al. (1993) Residues in the TATA-binding protein required to mediate a transcriptional response to retinoic acid in EC cells. *Nature* 365: 562-566.
12. Pugh BF, Tjian R (1990) Mechanism of transcriptional activation by Sp1: evidence for coactivators. *Cell* 61: 1187-1197.
13. Strubin M, Struhl K (1992) Yeast and human TFIID with altered DNA-binding specificity for TATA elements. *Cell* 68:721-730.
14. Nordeen SK, Moyer ML, Bona BJ (1995) Modulation of glucocorticoid-regulated transcription by purines: novel characteristics and implications for tissue specificity of steroid responses. *Endocrinology* 136: 1120-1127.
15. Beck CA, Weigel NL, Moyer ML, et al. (1993) The progesterone antagonist RU486 acquires agonist activity upon stimulation of cAMP signaling pathways. *Proc Natl Acad Sci U S A* 90: 4441-4445.
16. Nordeen SK, Bona BJ, Moyer ML (1993) Latent agonist activity of the steroid antagonist, RU486, is unmasked in cells treated with activators of protein kinase A. *Mol Endocrinol* 7: 731-742.
17. Moyer ML, Borror KC, Bona BJ, et al. (1993) Modulation of cell signaling pathways can enhance or impair glucocorticoid-induced gene expression without altering the state of receptor phosphorylation. *J Biol Chem* 268: 22933-22940.
18. Nordeen SK, Moyer ML, Bona BJ (1994) The coupling of multiple signal transduction pathways with steroid response mechanisms. *Endocrinology* 134: 1723-1732.
19. Nordeen SK (1994) Potentiation of glucocorticoid-mediated induction of the mouse mammary tumor virus promoter in mammary carcinoma cells by activation of protein kinase C. *Biochem Biophys Res Commun* 198: 1183-1188.
20. Dutertre M, Smith CL (2003) Ligand-independent interactions of p160/steroid receptor coactivators and CREB-binding protein (CBP) with estrogen receptor-alpha: regulation by phosphorylation sites in the A/B region depends on other receptor domains. *Mol Endocrinol* 17: 1296-1314.
21. Wu RC, Smith CL, O'Malley BW (2005) Transcriptional regulation by steroid receptor coactivator phosphorylation. *Endocr Rev* 26: 393-399.
22. Kato S, Masuhiro Y, Watanabe M (2000) Molecular mechanism of a cross-talk between oestrogen and growth factor signaling pathways. *Genes Cells* 5: 593-601.
23. Kit S, Dubbs DR, Piekarksi LJ, et al. (1963) Deletion of thymidine kinase activity from L cells resistant to bromodeoxyuridine. *Exp Cell Res* 31: 297-312.
24. Ralph P, Moore MA, Milsson K (1976) Lysozyme synthesis by established human and murine histiocytic lymphoma cell lines. *J Exp Med* 143: 1528-1533.
25. Gatlin J, Campbell LH, Schmidt MG, et al. (1995) Direct-rapid (DR) mutagenesis of large plasmids using PCR. *Biotechniques* 19: 559-564.
26. Nordeen SK (1988) Luciferase reporter gene vectors for analysis of promoters and enhancers. *Biotechniques* 6: 454-458.
27. Lieberman BA, Bona BJ, Edwards DP, et al. (1993) The constitution of a progesterone response element. *Mol Endocrinol* 7: 515-527.
28. Biggs JR, Ahn NG, Kraft AS (1998) Activation of the mitogen-activated protein kinase pathway in U937 leukemic cells induces phosphorylation of the amino terminus of the TATA-binding protein. *Cell Growth Differ* 9: 667-676.
29. Fujimoto N, Katzenellenbogen BS (1994) Alteration in the agonist/antagonist balance of antiestrogens by activation of protein kinase A signaling pathways in breast cancer cells: antiestrogen selectivity and promoter dependence. *Mol Endocrinol* 8: 296-304.

30. Keenan CR, Mok JS, Harris T, et al (2014) Bronchial epithelial cells are rendered insensitive to glucocorticoid transactivation by transforming growth factor-β1. *Respiratory Res* 15: 55
31. Piovan E, Yu J, Tosello V, et al. (2013) Direct reversal of glucocorticoid resistance by AKT inhibition in acute lymphoblastic leukemia. *Cancer Cell* 24: 766-776.
32. Beck IM, Vanden Berghe W, Vermeulen L (2009) Crosstalk in inflammation: the interplay of glucocorticoid receptor-based mechanisms and kinases and phosphatases. *Endocr Rev* 30: 830-882.
33. Kumar R, Calhoun WJ (2008) Differential regulation of the transcriptional activity of the glucocorticoid receptor through site-specific phosphorylation. *Biologics* 2: 845-854.
34. Ward RD, Weigel NL (2009) Steroid receptor phosphorylation: Assigning function to site-specific phosphorylation. *Biofactors* 35: 528-536.
35. Chen W, Dang T, Blind RD, et al. (2008) Glucocorticoid receptor phosphorylation differentially affects target gene expression. *Mol Endocrinol* 22: 1754-66.
36. Espinas ML, Roux J, Pictet R, et al. (1995) Glucocorticoids and protein kinase A coordinately modulate transcription factor recruitment at a glucocorticoid-responsive unit. *Mol Cell Biol* 15: 5346-5354.
37. Rowan BG, Garrison N, Weigel NL, et al. (2000) 8-Bromo-cyclic AMP induces phosphorylation of two sites in SRC-1 that facilitate ligand-independent activation of the chicken progesterone receptor and are critical for functional cooperation between SRC-1 and CREB binding protein. *Mol Cell Biol* 20: 8720-8730.
38. Johnson AB, O'Malley BW (2012) Steroid receptor coactivators 1, 2, and 3: critical regulators of nuclear receptor activity and steroid receptor modulator (SRM)-based cancer therapy. *Mol Cell Endocrinol* 348: 430-439.
39. Das D, Scovell WM (2001) The binding interaction of HMG-1 with the TATA-binding protein/TATA complex. *J Biol Chem* 276: 32597-32605.
40. Boonyaratanakornkit V, Melvin V, Prendergast P, et al. (1998) High-mobility group chromatin proteins 1 and 2 functionally interact with steroid hormone receptors to enhance their DNA binding *in vitro* and transcriptional activity in mammalian cells. *Mol Cell Biol* 18: 4471-4487.
41. Melvin VS, Edwards DP (1999) Coregulatory proteins in steroid hormone receptor action: the role of chromatin high mobility group proteins HMG-1 and -2. *Steroids* 64: 576-586.
42. Hernandez N (1993) TBP, a universal eukaryotic transcription factor? *Genes Dev* 7:1291-1308.
43. Mittal V, Hernandez N (1997) Role for the amino-terminal region of human TBP in U6 snRNA transcription. *Science* 275: 1136-1140.
44. Mittal V, Ma B, Hernandez N (1999) SNAP(c): a core promoter factor with a built-in DNA-binding damper that is deactivated by the Oct-1 POU domain. *Genes Dev* 13: 1807-1821.
45. Zhao X, Herr W (2002) A regulated two-step mechanism of TBP binding to DNA: a solvent-exposed surface of TBP inhibits TATA box recognition. *Cell* 108: 615-627.
46. Um M, Yamauchi J, Kato S, et al. (2001) Heterozygous disruption of the TATA-binding protein gene in DT40 cells causes reduced cdc25B phosphatase expression and delayed mitosis. *Mol Cell Biol* 21: 2435-2448.
47. Schmidt EE, Bondareva AA, Radke JR, et al. (2003) Fundamental cellular processes do not require vertebrate-specific sequences within the TATA-binding protein. *J Biol Chem* 278: 6168-6174.

48. Hobbs NK, Bondareva AA, Barnett S, et al. (2002) Removing the vertebrate-specific TBP N terminus disrupts placental beta2m-dependent interactions with the maternal immune system. *Cell* 110: 43-54.