The Minimal Transactivation Domain of the Basic Motif-Leucine Zipper Transcription Factor NRL Interacts with TATA-binding Protein*

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The basic motif-leucine zipper (bZIP) transcription factor NRL controls the expression of rhodopsin and other phototransduction genes and is a key mediator of photoreceptor differentiation. To delineate the molecular mechanisms underlying transcriptional initiation of rod-specific genes, we characterized different regions of the NRL protein using yeast-based autoactivation assays. We identified 35 amino acid residues in the proline- and serine-rich N-terminal region (called minimal transactivation domain, MTD), which, when combined with LexA or Gal4 DNA binding domains, exhibited activation of target promoters. Because this domain is conserved in all proteins of the large Maf family, we hypothesized that NRL-MTD played an important role in assembling the transcription initiation complex. Our studies showed that the NRL protein, including the MTD, interacted with full-length or the C-terminal domain of TATA-binding protein (TBP) in vitro. NRL and TBP could be co-immunoprecipitated from bovine retinal nuclear extract. TBP was also part of c-Maf and MafA (two other large Maf proteins)-containing complex(es) in vivo. Our data suggest that the function of NRL-MTD is to activate transcription by recruiting or stabilizing TBP (and consequently other components of the general transcription complex) at the promoter of target genes, and a similar function may be attributed to other bZIP proteins of the large Maf family.

Initiation of transcription is a major target for genetic regulation of diverse biological processes, such as development and homeostasis (1). Transcriptional regulation is mediated by interactions among a plethora of proteins, which include chromatin-modifying proteins, activators, repressors, TATA-binding protein (TBP)-associated factors, and basal transcription factors (2). Chromatin-modifying proteins and transcriptional activators bind to the promoter and form a DNA-protein complex, termed “enhancesome” (3, 4). Basal transcription factors, either pre-assembled with RNA polymerase II or in a stepwise manner, bind to the enhancesome to produce the pre-initiation complex (5). The basal transcription factors (TFIIA, TFIIB, TFIID, TFIIIE, TFIIF, and TFIHH) generally include a complex of proteins; of these, TFIIH contains the TATA box-binding protein, TBP (6). Once a stable pre-initiation complex is formed, the gene transcription is initiated.

Tissue-or cell-type-specific expression of genes is regulated, to a large extent, by transcription factors that can act either as activators or repressors (7). Transcription factors are modular proteins and typically contain a DNA binding domain (DBD) and at least one transactivation domain (8). At least twelve different DBDs and three types of activation domains have been described in mammalian systems (9, 10). The bZIP family of transcription factors contains a motif rich in basic amino acid residues that bind to DNA and a leucine zipper domain that is involved in homo- or heterodimerization (11). The Maf proteins constitute a subfamily within bZIP proteins and are important mediators of gene regulation in diverse cell types (12, 13). The small Maf proteins (MafF, MafG, and MafK) lack a transactivation domain and can form heterodimers with cap ‘n’ collar (CNC) or Bach family proteins. In contrast, large Maf proteins (c-Maf, MafB, MafA or L-Maf, and NRL) have a larger N-terminal region that acts as a transactivation domain; c-Maf and MafB are widely expressed, whereas MafA and NRL exhibit restricted expression in different parts of the eye (12, 13).

Multiple phosphorylated isoforms of NRL are expressed in rod and not cone photoreceptors (14, 15). Rhodopsin is the photopigment in rods, and its expression is stringently controlled at the level of transcription (16–19). The promoter region of the rhodopsin gene includes binding sites for several transcription factors, such as NRL and CRX (cone rod homeobox) (20, 21). NRL can interact with CRX, NR2E3, and other proteins and synergistically activate (or modulate) the expression of rhodopsin and several rod-specific genes (20–26). Consistent with its role, mutations in the human NRL gene are

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1 The abbreviations used are: TBP, TATA-binding protein; CRX, cone-rod homeobox; DBD, DNA binding domain; MTD, minimal transactivation domain; NRL, neuronal retina leucine zipper; RNE, retinal nuclear extract; X-gal, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside; WT, wild type; CT, C-terminal; NT, N-terminal; MAPK, mitogen-activated protein kinase.
associated with autosomal dominant retinitis pigmentosa (27–29). In mice, loss of NRL results in a complete loss of rods and a concomitant increase in functional S-opsin-expressing cones (30).

Although the function of DBDs has been investigated in great depth, the transactivation domains of transcription factors are less well characterized. To better understand the biochemical mechanisms underlying rod-specific gene regulation, we are interested in dissecting the role of distinct domains of NRL and evaluate their interactions with other proteins of the transcriptional machinery. To date, the transactivation domain of NRL has been broadly defined as a proline- and serine-rich 35-amino acid domain in the N-terminal region of NRL that has been broadly defined as a proline- and serine-rich region upstream of the C-terminal bZIP domain (20). Here, we report that a proline- and serine-rich 35-amino acid domain in the N-terminal region of NRL is sufficient for transactivation of target promoters. We show that the region, including this minimal transactivation domain (MTD), can interact with TBP in vitro and that NRL and TBP are part of the same protein complex in vivo. We demonstrate that TBP is also part of multiprotein complexes containing two other large Maf proteins, c-Maf and MaFα, in vivo. Our results suggest that NRL-MTD recruits or stabilizes the basal transcription factors associated with autoregulatory mechanisms.

**EXPERIMENTAL PROCEDURES**

**NRL Constructs**—Primers with EcoRI or NotI restriction sites were designed to amplify and generate NRL deletions D1–D11 and internal deletions D1–D11 and internal deletions D4–D5, respectively. Inserts were cloned into pcDNA4c and completely sequenced.

**Yeast Transformations**—Yeast strains used in the study were L40 (22) for the pHyblexZeo vector, and Y187 (MATa, ura3–52, his3–200, ade2–101, trp1–901, leu2–3,112, gal4D, met15Δ, his3–200, URA3–GAL1 TATA-lacZ) for the pGBKT7 vector. Yeast transformations were performed as described (31) and plated onto YPD (yeast extract, peptone, glucose, adenine, and arginine) and -Trp plates for the pGBKT7 vectors, respectively. Individual colonies were then examined for autoactivation.

**Autoactivation**/β-Galactosidase Tests—Colonies were patched onto an appropriate selection plate (His+, +20 μg/ml Zeocin, +50 μg/ml 3-amino-triazole for pHyblexZeo vectors and -Trp plates for the pGBKT7 constructs) and with and without 50 μg/ml X-gal, previously spread over the plate. The plates were incubated at 30 °C for 3–7 days. Yeast colonies on plates containing X-gal were analyzed for color change to blue, thereby showing autoactivation of the lacZ reporter. Patch after plates without X-gal were examined using a β-galactosidase filter assay, as described in the Hybrid Hunter Manual (Invitrogen).

**β-Galactosidase liquid assays** were performed as described (32). Briefly, one yeast colony from each construct was grown in 10 ml of YPD culture overnight. Triplicate dilutions of cultures were made at an A600 of 0.25 in 2 ml of YPD plus Zeocin (except L40 control, where only YPD was used), prior to growth to log phase. A600 of aliquots of each culture were measured after 3 h growth at 30 °C. Cells were quickly centrifuged at 16,000 g for 1 min, and the cell pellets were each resuspended in 500 μl of Z-buffer (32). Each sample was subsequently treated to three freeze thaw cycles and then incubated at 30 °C for 1 h. The samples were divided into two plates containing X-gal and were analyzed for color change.

**Immunoprecipitation from Bovine Retinal Nuclear Extract and Other Tissues**—Retinal nuclear extract (RNE) was prepared according to the published protocol (24). Mouse pancreatic total protein extract and bovine kidney extract were prepared in 1× phosphate-buffered saline containing protease inhibitors. For immunoprecipitation, 300 μg of protein was incubated with anti-NRL (15), anti-TBP, anti-e-Maf, or anti-MaFα antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) over-
night at 4 °C with gentle shaking. At the end of incubation, protein-A- or protein-G-agarose beads were added to precipitate the complex. The beads were washed with phosphate-buffered saline containing 1% Triton X-100. The protein was suspended in Laemmli sample buffer and then analyzed by SDS-PAGE followed by immunoblotting using the appropriate antibodies (24).

**RESULTS**

**Identification of NRL Domains Capable of Transactivation, Using Yeast Autoactivation Assays**—NRL bait constructs that include LexA or Gal4 DNA binding domains would be capable of binding to the promotor of an auxotrophic and/or LacZ marker in an appropriate yeast strain. If a protein fragment contained a transactivation domain, it would be expected to autoactivate and turn on the appropriate reporter genes in yeast, as illustrated earlier (35, 36). We had previously shown that full-length NRL activated rhodopsin promoter, whereas DD10, a naturally occurring variant of NRL that lacked most of the proline- and serine-rich region, did not (20). Initially, bait constructs with different regions of NRL were generated with LexA DBD and examined by yeast autoactivation assay using WT and DD10 as positive and negative controls, respectively (Fig. 1). L40 yeast patches on a plate with X-gal showed autoactivation with construct D1 (amino acids 30–237). The removal of N-terminal 74 amino acids (construct D2) dramatically reduced the reporter gene activity. Filter lift assays, a more sensitive test of β-galactosidase activity, demonstrated that the D2 construct (residues 75–237) did reveal β-galactosidase activity. The reduction in transactivation was confirmed by yeast liquid assays (Fig. 1). N-terminal deletions beyond amino acids 75 (D3, D4, and D5) resulted in the elimination of autoactivation capability, as determined by the three β-galactosidase assays. NRL-D6, encoding only residues 1–57, completely lacked transactivation activity, whereas internal fragments containing amino acids 30–93 (D7, D8, and D9) were able to activate the reporter genes. Notably, D7 (amino acids 1–123) and D9 (amino acids 30–143) with 50 additional amino acids compared with D9, showed greatly reduced levels of autoactivation. These results suggest that the residues from 75 to 143 may have additional domains that can affect the degree of NRL transactivation. Constructs D10 (amino acids 88–143) and D11 (amino acids 122–181) did not show autoactivation. Hence, the transactivation ability of NRL was primarily contained within the residues 30–93.

To define the minimal transactivation domain (MTD), we

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**FIG. 1. Yeast autoactivation experiments on NRL N-terminal deletions and internal fragments.** Full-length NRL, DD10, and deletions D1 through D11 were examined for autoactivity when placed within the LexA DNA binding domain (DBD)-containing pHyLexZeo vector. The name of each construct, the NRL amino acid region studied, and a schematic of the NRL protein, including the extended homology domain (EHD), basic domain (BD) and leucine zipper domain (L. Zipper) are shown. The LexA DBD in each construct is not shown in the schematic. Yeast patched in the presence of 50 mM 3-aminotriazole (3AT) with X-gal on the plate is shown in the center column. To the right is a second set of patches grown without X-gal and examined using the filter lift assay. On the far right are the amounts of β-galactosidase (β-gal) units for L40 (as a negative control) and each of the transformed yeast colonies.
examined additional constructs. NRL regions 30–74 and 40–74 showed transactivation activity close to the wild type NRL levels (Fig. 2A). The constructs with NRL region 30–64 and with the region 58–74 did not exhibit any activation of reporter genes in yeast assays. In addition, we made constructs with NRL regions 75–93 and 75–102 to map the residual activity observed in D2 (amino acids 75–237); however, neither showed autoactivation. These data suggest that the domain responsible for the residual activity was either after amino acid 102 or was included in 75–102 but was disrupted and non-functional.

To establish that the primary region responsible for transactivation was contained within amino acids 30–74, we generated an in-frame deletion variant of NRL removing amino acids 30–74. This construct, NRL-Δ30–74, gave filter lift and liquid assay results similar to D2, confirming that the domain from residues 30–74 includes the major domain involved in the transactivation function of NRL.

NRL-MTD Can Function with the Gal4 DNA Binding Domain—Because the NRL region from residues 40–74 (hereafter called NRL-MTD) was sufficient for transactivation with LexA DBD, we examined whether it can function with another DNA binding domain. We transferred several NRL inserts into the pGBKT7 vector, which contains the Gal4 DNA binding domain, and examined their activity in Y187 yeast. The β-galactosidase activity of these constructs could not be observed on X-gal-containing -Trp plates (data not shown), suggesting that the level of transactivation of the β-galactosidase reporter in Y187 yeast is not as strong as in L40 yeast. Nevertheless, the filter lift assay showed autoactivation by full-length NRL, NRL 30–93, and NRL-Δ30–74, whereas weaker activity was detected with NRL-Δ30–74 (Fig. 3).

NRL-MTD Is Conserved in Other Large Maf Proteins—To examine whether NRL-MTD exhibits similarity to the putative transactivation domains in other Maf proteins, we performed

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**Fig. 2.** Determination of the minimal domain required for NRL autoactivation. **Top panel:** NRL cDNA regions encoding smaller amino acid domains in the pHybLexZeo vector are shown in the schematic on the far left. Yeast colonies patched in the presence of 50 mM 3-aminotriazole and examined using the filter lift assays are shown. On the right are the β-galactosidase units for each transformed construct in L40 yeast from the liquid assay. **Bottom panel:** schematic of full-length NRL, DD10, and NRL-Δ30–74. All three constructs were in the pHybLexZeo vector. Yeast patches, filter lift assays, and β-galactosidase liquid assay tests are shown as above.
homology searches. A ClustalW alignment of NRL-MTD with c-MAF, MAFA, and MAFB (three large Maf proteins) showed a high degree of similarity, particularly for the conservation of serine and proline residues (Fig. 4).

NRL and TBP Can Interact in Vitro—The high degree of sequence conservation suggested that the MTD of NRL (and of other large Maf proteins) must have an important fundamental function. We therefore examined whether NRL-MTD can interact with components of the basal transcriptional machinery. In the rhodopsin promoter, the NRL-response element (NRE) is close to the TATA-box (22). Assays involving in vitro transcription/translation followed by immunoprecipitation were first performed to test the interaction of different NRL regions with TBP (Fig. 5). Full-length TBP could be co-immunoprecipitated with full-length NRL or with NRL amino acids 30–93 using anti-Xpress tag antibody (Fig. 5A). The NRL regions from 30–74 or 40–74 were not tested, because a sufficient amount of protein could not be produced for the assay (data not shown).

We examined the N-terminal (amino acids 1–161, containing the polyglutamine domain; TBP-NT) and the C-terminal (amino acids 162–339; TBP-CT) regions of TBP for their interaction with NRL (Fig. 5B). Our results show that TBP-CT could be co-immunoprecipitated with NRL by the anti-NRL antibody, whereas TBP-NT was not. As expected, anti-Xpress tag antibody and anti-TBP antibody did not immunoprecipitate TBP and NRL, respectively (Fig. 5A).

NRL and TBP Can Interact in Vivo—To validate the in vivo significance of NRL-TBP interaction, we performed co-immunoprecipitation experiments using the bovine RNE with anti-NRL or anti-TBP antibodies. Immunoblot analysis of the RNE immunoprecipitate showed a band of the expected size (Fig. 6, C and D). TBP was not immunoprecipitated in the absence of primary antibody. The large Maf protein MafB could not be tested for its interaction with TBP, because a sufficient amount of MafB protein was not detected in tissues that were examined.

NRL May Interact with TBP in an Extended Conformation—A manual sequence alignment based upon the Brf1-TBP x-ray structure (33) was performed, and a homology model was calculated (Fig. 7). The model yields a structurally sensible homology model and displays plausible chemical interactions. Due to the low sequence similarity (12.5% identity) and the extended conformation of the Brf1 template structure, the relative positioning of the NRL-Brf1 sequence alignment may differ in detail from the model obtained in vivo. However, we believe the functional implications of this model are strongly supported by the Brf1-TBP x-ray structure, the functional data with NRL, and the sequence similarity between Brf1 and NRL.

The homology model is consistent with the predicted electrostatic interactions between TBP and NRL, similar to TBP-Brf1 interactions seen in the experimental structure (33). Positively charged residues Arg-107, Lys-133, Arg-137, Lys-138, Arg-141,
Interaction of NRL with TBP in vitro

Interaction of NRL with TBP was studied by in vitro transcription and translation of the respective polypeptides followed by co-immunoprecipitation, as described under “Experimental Procedures.”

A, interaction of [35S]-TBP with Xpress-tagged full-length NRL and NRL 30–93. Immunoprecipitation was done using the anti-Xpress tag antibody. Lanes are: 1, in vitro translation input (5%) for [35S]-TBP; 2, [35S]-TBP and NRL; 3, [35S]-TBP and NRL 30–93; 4, [35S]-TBP and NRL immunoprecipitated without anti-Xpress tag antibody (control); 5, [35S]-TBP and NRL 30–93 immunoprecipitated without anti-Xpress tag antibody (control); 6 and 7, in vitro translation inputs for [35S]-NRL 30–93 and [35S]-NRL, respectively; 8, in vitro translation input for [35S]-TBP; 9, [35S]-TBP immunoprecipitated with anti-Xpress tag antibody; 10, in vitro translation input for [35S]-NRL; 11, [35S]-NRL immunoprecipitated with anti-NRL antibody (control).

B, interaction of different domains of TBP with full-length NRL. N-terminal (TBP-NT) and C-terminal (TBP-CT) domains of TBP were in vitro transcribed and translated in the presence of [35S]methionine and incubated with non-radioactive in vitro translated NRL followed by immunoprecipitation using anti-NRL antibody. Lanes are: 1 and 2, in vitro translated [35S]-TBP-NT and [35S]-TBP-CT, respectively; 3, [35S]-TBP and NRL; 4, [35S]-TBP-NT and NRL; 5, [35S]-TBP-CT and NRL; 6, [35S]-TBP-NT and NRL immunoprecipitated without anti-NRL antibody (control); 7, [35S]-TBP-CT and NRL immunoprecipitated without anti-NRL antibody (control).
and Lys-151 are presumed to interact with a subset of predicted phosphoserine residues Ser-38, Ser-46, Ser-50, and Ser-54 (Fig. 7). In the model, Ser-50 and Ser-54 are located in the positively charged region of TBP and are able to form salt bridge type interactions with arginine and lysine residues that are involved in the TBP/Brf1 complex. Ser-54 of NRL is within interaction distance of Lys-138 and Arg-107 of TBP, Ser-50 is near Arg-141 and Lys-145, and Ser-46 is near Arg-137, Lys-133, and Lys-151. The model places residues 28–63 of NRL in a positively charged region of TBP and places residues Ser-38 to Glu-55 of NRL in immediate proximity to three arginine and four lysine residues. The predicted salt bridge interactions between NRL and TBP are analogous to the Brf1-TBP interactions seen experimentally (33).

DISCUSSION

How combinatorial and cooperative action(s) of transcriptional activators mediate cell-type-specific expression of target genes has been under intense scrutiny. Pioneering studies in bacteria and yeast strongly advocated the recruitment model, which stipulated that activator proteins bring the basal transcription factors to the promoter regions (37). The basic tenets of this model are valid even in higher eukaryotes; however, the underlying molecular mechanisms appear to be diverse and complex. To initiate the transcription of a particular gene, activator proteins must orchestrate the assembly of a stable complex of numerous proteins in a stepwise manner (2, 5). Hence, the one or more transcriptional activation domains of activator proteins are expected to interact with distinct modulators and an abundance of general transcription factors. A better understanding of cell-type-specific gene expression will therefore require a precise delineation of these interactions. Here, we have used yeast autoactivation assays to identify the minimal domain of NRL (residues 40–74) that is sufficient for activating transcription. NRL-MTD of 35 amino acids is conserved in other proteins of the large Maf family. In vitro and in vivo co-immunoprecipitation studies strongly suggest that this region is involved in interactions with TBP. We demonstrate that other large Maf proteins also exist in the TBP-containing multiprotein complex(es) in vivo. Our results, therefore, provide a mechanistic framework for assembling a stable transcription complex at the promoters of target genes of NRL and other large Maf proteins.

Photoreceptors are among the most metabolically active post-mitotic cells; ~9 billion molecules of opsin are produced every second in each human retina (38). The expression of opsins and other phototransduction proteins must, nevertheless, be tightly controlled; defects in rhodopsin or its over- or under-expression have been shown to result in photoreceptor degeneration (39–41). NRL is the key transcriptional regulator in developing and mature rod photoreceptors (20, 27, 30, 42). It interacts with CRX, Fiz1, NR2E3, and other transcription factors and activates the expression of many rod-specific genes, such as rhodopsin (20–26). It is expected that a transcriptional activator, such as NRL, would make multiple contacts in the initiation complex; however, this is the first report that delineates the involvement of a 35-residue domain within the pro-
line- and serine-rich transactivation region of NRL in interactions with TBP, a key component of the basal transcriptional machinery. Because the MTD of NRL is conserved among the large Maf protein family members, it is reasonable to hypothesize that all large Maf proteins can interact with TBP. Our results strongly suggest that c-Maf and MafA can also interact with TBP in vivo thereby strengthening the model for transcriptional activation mediated by the large Maf proteins. Our studies provide strong support for the prevailing models of transcriptional activation (1, 5, 37, 43–45).

Although smaller MTDs of 17 and 33 residues have been reported for Gal4p and Gln3p, respectively (46, 47), transactivation domains of mammalian activators are reported to be large (48–50). Using c-myc as a model, it has been suggested that a large transactivation domain is needed for interactions with TBP (51). NRL-MTD of 35 amino acids is, therefore, one of the smallest stretches of residues in a mammalian protein that has been shown to activate transcription. In addition, we were able to demonstrate the interaction of NRL residues 30–93 (64 amino acids), which include the MTD, with TBP by in vitro immunoprecipitation experiments.

We have attempted to construct a model of NRL-MTD interaction with TBP C-terminal domain, based upon the experimentally determined structure of TBP and Brf1 (33). The TBP-Brf1 model proposes three electrostatic interactions between four aspartic acid residues of Brf1 and a highly positively charged region of TBP: Arg-137/Asp-464, Lys-138/Asp-467, Arg-141/Asp-466, and Lys-145/Asp-467 (TBP residues are named first) (33). Our model supports the notion that negatively charged phosphoserine residues of NRL could interact with TBP in an analogous fashion to the aspartic acids of Brf1. Within the 30–93-amino acid region of NRL, there are several conserved serine and threonine residues, which are potential phosphorylation sites for MAPK (52–54) and glycogen synthase kinase 3β (55, 56). It should be noted that MAPK pathways are of central importance in controlling rod photoreceptor survival, and it is possible that such processes are mediated via NRL modulation (57). Phosphorylation of some or all of the serine and threonine residues within the 30–93 region could be critical for salt bridge formation between NRL and TBP. Although we observed TBP interaction with in vitro synthesized NRL region 30–93, we hypothesize that differential phosphorylation (as observed for NRL in vivo (15)) of conserved serine residues in NRL could modulate the extent of its productive interaction with TBP and other transcription factors. This may, in turn, provide a convenient mechanism for precisely fine-tuning the expression of rhodopsin and other rod-specific genes. This hypothesis is consistent with the human mutation reports; 5 of the 6 mutations of NRL, so far reported in patients with autosomal dominant retinitis pigmentosa, are at residues Ser-50 and Pro-51 (27–29), and the S50T mutation was shown to enhance the ability of NRL to transactivate the rhodopsin promoter in the presence of CRX (27). In addition, the identification of phosphorylation and subsequent mutation analysis of residue Ser-65 in MafA, the homologous residue of Ser-50 in NRL, further supports this hypothesis (58, 59). To investigate it further, we altered individual serine and threonine residues (Ser-38, Ser-41, Thr-42, Ser-45, Ser-50, Thr-52, and Ser-54) to alanine and examined their effect on the transactivation ability of NRL using the rhodopsin promoter activity assay similar to that reported earlier (27). Although we observed altered NRL synergy with CRX at various DNA concentrations, the individual changes as a whole did not significantly alter the activity of NRL (data not shown). We suggest that the change from serine to alanine in the residues tested may not significantly alter the conformation of NRL and that multiple modifications (as shown in Refs. 58 and 59) may be required to elicit a more significant functional effect on transactivation. Additional studies will be required to further characterize the role of individual amino acid residues that constitute the MTD.

One potential use of delineating the MTD is in the development of an artificial transcription factor, which can be utilized for therapeutic purposes by specifically activating or inhibiting the target genes (60). NRL-MTD of 35 amino acids is perhaps one of the shortest transactivation domains defined for a mammalian transcription factor. Because it can function in yeast with two different DBDs and appears to be involved in TBP interaction, we suggest that this domain can be used for activating gene expression in a variety of cell types. It should also be possible to modify various residues of NRL-MTD and alter its phosphorylation status to fine-tune its activity on target promoters.

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FIG. 7. Homology model of a putative NRL-TBP complex. NRL is shown in space-filling format and TBP (blue) is shown in ribbon format. NRL adopts an extended conformation that forms an extensive interaction surface with TBP. Residues 41–65 interact with a positively charged region of TBP that has been shown to be important in transactivator binding to TBP (33). The phosphorylated residues Ser-50 and Ser-54 are postulated to interact with Arg-137 and Arg-141, respectively.
Interaction of NRL Minimal Transactivation Domain with TBP