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Controlled transcriptional regulation in eukaryotes by a novel transcription factor derived from *Escherichia coli* purine repressor☆,☆☆

Eun-Hee Yeon,[a] Ju-Young Noh,[a] Jong-Min Kim,[a] Min-Young Lee,[a] Sarah Yoon,[a] Sang-Kyu Park,[a] Kang-Yell Choi,[b] and Kyung-Sup Kim[a,*]

[a] *Department of Biochemistry and Molecular Biology, Institute of Genetic Science, Yonsei University, College of Medicine, 134 Shinchon-dong, Seodaemun-gu, Seoul 120-752, Republic of Korea*
[b] *Department of Biotechnology, Yonsei University College of Engineering, 134 Shinchon-dong, Seodaemun-gu, Seoul, Republic of Korea*

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Abstract

Unlike the DNA-binding domains (DBD) of most eukaryotic transcription factors, *Escherichia coli* LacI family transcription factors are unable to bind to specific target DNA sequences without a cofactor-binding domain. In the present study, we reconstructed a novel DBD designated as PurHG, which binds constitutively to a 16 bp purine repressor operator, by fusion of the purine repressor (PurR) DBD (residues 1–57) and the GAL4 dimerization domain (DD, residues 42–148). Binding of PurHG to DNA requires the dimerization and a hinge helix of PurR DBD. When the PurHG was expressed as a fusion protein in a form of a transcription activator (PurAD) or an artificial nuclear receptor (PurAPR or PurAER) responding to ligand, such as RU486 or β-estradiol, it could regulate the expression of the reporter genes in NIH3T3 cells. The prerequisite region of the GAL4 DD for DNA-binding was amino acid residues from 42 to 98 in the form of PurAD, while the amino acid residues from 42 to 75 were sufficient for ligand-dependent regulation in the form of PurAPR. These results suggest that the dimerization function of the progesterone ligand-binding domain could be substituted for region 76–98 of the GAL4 DD. In summary, the fusion of the PurR DBD and the GAL4 DD generates fully active DNA-binding protein, PurHG, in vitro and in vivo, and these results provide the direct evidence of structural predictions that the proximate positioning of PurR hinge helical regions is critical for DNA-binding.

Keywords: Purine repressor; Lactose repressor; Artificial transcription factor; Nuclear receptor; Progesterone receptor; Estrogen receptor

Tightly controlled gene expression systems with defined target specificities could provide invaluable tools for basic and applied researches. Introducing prokaryotic transcription factors in mammalian cells is particularly attractive because it could provide tight gene regulation in eukaryotic cells, while eukaryotic transcription factors affect the expression of endogenous genes by binding to the consensus sequences. GAL4 [1–3] and LexA [4] are the representative transcription factors of prokaryotes or yeast, which have been used for development of eukaryotic transcription activators.

Escherichia coli purine repressor (PurR) is a dimeric transcription regulator, which belongs to the LacI family [5–7]. The biochemical and the structural studies have shown that PurR consists of an NH_2-terminal DNA-binding domain (DBD) (residues 3–56) and a COOH-terminal corepressor-binding domain (CBD) (residues 60–341) [6–8]. PurR DBD consists of helix–turn–helix and hinge helix motifs, and PurR CBD is responsible for the corepressor-binding and dimerization [6,7]. Binding of corepressors to PurR under purine

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☆☆ *Abbreviations:* DBD, DNA-binding domain; CBD, corepressor-binding domain; AD, activation domain; LBD, ligand-binding domain; DD, dimerization domain; PurR, purine repressor; PuRE, PurR operator element; GST, glutathione *S*-transferase; EMSA, electrophoretic gel mobility shift assay.

* Corresponding author. Fax: +82-2-312-5041.
E-mail address: kyungsup59@yumc.yonsei.ac.kr (K.-S. Kim).

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rich condition acts as an environmental switch to stop de novo synthesis of purine nucleotides. An isolated PurR DBD is unable to specifically bind to DNA by itself. However, in an intact PurR, the CBD undergoes a conformational change when corepressors bind to it, which stabilizes the interaction between the hinge helix of DBD and the minor groove of the operator [6–8]. It then relocates the helix–turn–helix motif into a position which allows it to bind to the successive major groove [6,7]. The hinge helix of PurR is essential for DNA-binding ability of PurR and that was demonstrated by mutational analysis [9] and structural studies using NMR and X-ray crystallography [6,10]. The hinge helix in a corepressor-bound dimerized PurR has an ordered α-helix, while the hinge helix of an isolated free DBD is disordered [5,10]. The crucial requirement of an ordered hinge helix have hindered PurR DBD from being developed as an artificial gene regulator.

In the present work, we have generated a novel artificial transcription factor, derived from PurR DBD and GAL4 DD. The hybrid protein is designated as PurHG, which is able to bind specifically to the corresponding operator sequences, regardless of corepressors. By fusing this construct to activation domain (AD) of SREBP-1a [11–13] and ligand-binding domains (LBD) of nuclear receptors such as progesterone and estrogen receptors [14], we developed tight controllable mammalian artificial transcription activators.

Materials and methods

Recombinant proteins of PurR DBD. The PurR gene, which contains the coding region corresponding to amino acid 1–155 of *E. coli* PurR, was amplified from pPR1010 plasmid [5] by polymerase chain reaction (PCR) and cloned into *Bam*HI site of pGEX-4T1 vector (Amersham–Pharmacia Biotech), resulting in pGEX-Pur155. The PurG cDNA, which is the hybrid sequence for residues 1–48 of PurR DBD and residues 42–148 of GAL4 DD, was generated by PCR as following procedures. The cDNA for amino acids 1–48 of PurR DBD, was amplified from pPR1010 plasmid, using Pur-s primer (5′-CTG AGA TCT AAT ACC ATG GCA ACA ATA-3′) and Pur-as1 primer (5′-CCT TTT GGT TTT GGG GCT AGG GGA GTA GTG TAA TT-3′). The cDNA for the Gal4 DD (residues 42–148) was amplified from pFA2-Elk1 (Stratagene), using Gal4-s1 primer (5′-CAC TAC TCC CCT AGC CCC AAA ACC AAA AGG TCT CC-3′) and Gal4-as primer (5′-TGA AAA AGT GGA TCC GGG CGA TAC AGT-3′). Thirty nucleotides of 3′ sequences of the Pur-as1 primer and the Gal4-s1 primer were complementary to each other. Therefore, 30 bp sequence of 3′ end of amplified PurR cDNA is identical to that of 5′ end of GAL4 DD cDNA, and full length of PurG cDNA could be synthesized through the annealing and extension at the ends of cDNAs. Amplification of PurG cDNA was performed, using the mixture of above amplified PurR cDNA and GAL4 DD cDNA as a template with the primer set of Pur-s and Gal4-as. The cDNA for PurG was inserted into *Bam*HI site of pGEX4T1, resulting in pGEX-PurG. The construct of pGEX-PurHG, which contains cDNA hybrid for PurR DBD (residues 1–57) and the GAL4 DD (residues 42–148), was generated by the same procedures as pGEX-PurG, except for the use of Pur-as2 (5′-CCT TTT GGT TTT GGG GTT AAC CTT AAC CTT CAG GCT ACG CG-3′) and Gal4-s2 primer (5′-AGC CTG AAG GTT AAC CCC AAA ACC AAA AGG TCT CC-3′) instead of Pur-as1 and Gal4-s1 primers. For purification of recombinant proteins, the bacteria freshly transformed with each expression vector were grown to mid-log phage, and GST fusion proteins were induced for 4 h with 1 mM isopropyl-β-D-thiogalactopyranoside. The recombinant proteins were purified by glutathione–Sepharose 4B (Amersham–Pharmacia Biotech) affinity chromatography according to the manufacturer's protocol. The purity and concentration of the recombination proteins were verified by SDS–polyacrylamide gel electrophoresis followed by Coomassie brilliant blue staining.

Electrophoretic gel mobility shift assay (EMSA). The oligonucleotide probes were end-labeled with [γ-^{32}P]ATP (NEN Life Science Products) using T_4 polynucleotide kinase. The purified probes (0.1 pmol/μl) were incubated with purified recombinant PurR DBD proteins (0–500 ng) in a final volume of 20 μl, containing 10 mM Hepes, pH 7.9, 60 mM KCl, 1 mM EDTA, 5 mM dithiothreitol, 5 mM $MgCl_2$, 10% glycerol, 1 μg poly(dI–dC), and 0.5% BSA. After a 30 min incubation at room temperature, samples were resolved on a 4% polyacrylamide gel in 1× TBE (45 mM Tris, 45 mM boric acid, and 1 mM EDTA) at 200 V for 1 h. The gels were dried and exposed to X-ray film.

Construction of eukaryotic expression plasmids. The pCMV-GAL4APR was generated from pFA2-Elk1 (Stratagene) by inserting the cDNAs of progesterone receptor LBD and the SREBP-1a AD. First, the Elk1 region was replaced by a 0.2 kb *Bam*HI/*Sac*I fragment for SREBP-1a AD (residues 1–79) isolated from pET-SREBP-1a [15], resulting in pCMV-GAL4AD. Then the *Bam*HI/*Eco*RI fragment of progesterone receptor LBD (residues 645–891), which was amplified from RNA isolated from human breast cancer tissue, was inserted into *Bam*HI/*Eco*R sites between GAL4 DBD and SREBP-1a AD of pCMV-GAL4AD to generate pCMV-GAL4APR. The *Bgl*II/*Bam*HI fragment (520 bp) of PurHG cDNA amplified from pGEX-PurHG was subcloned into *Bam*HI site of pcDNA3 (Invitrogen) in proper orientation, to make pCMV-PurHG. Then the *Bam*HI/*Bgl*II fragment from pCMV-GAL4AD or pCMV-GAL4-APR, which corresponds to SREBP-1a AD or progesterone receptor LBD/SREBP-1a AD, was inserted into *Bam*HI site downstream of PurHG region of pCMV-PurHG, resulting in pCMV-PurAD and pCMV-PurAPR. To make the deletion constructs of GAL4 region, such as pCMV-PurAPRΔGal4, pCMV-PurAPR-Gal64, pCMV-PurAPR-Gal75, and pCMV-PurAPR-Gal98, a *Bam*HI restriction site was introduced in the GAL4 region of pCMV-PurAPR using the QuickChange site-directed mutagenesis kit (Stratagene), and then the *Bam*HI fragment from the newly introduced site to the 3′ end of the GAL4 dimerization element was removed. To generate pCMV-PurAER, the cDNA for the estrogen receptor LBD (residues 297–554) [14] was amplified from RNA isolated from human breast cancer tissue, which was used to replace the progesterone receptor LBD in pCMV-PurAPR. The reporter construct of pPuRE-luc was generated by inserting 5 repeats of PuRE sequence into the SacI site upstream of the modified rat ATP citrate-lyase minimal promoter (−60 to +67) [15], followed by the luciferase gene. The modified rat ATP citrate-lyase minimal promoter has a 'TATAAA' sequence at the −30/−25 site instead of the original 'GACAAA' sequence.

Cell culture and transfection assay. NIH3T3 cells obtained from ATCC were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin G sodium, and 100 μg/ml streptomycin sulfate. All cell culture materials were purchased from Life Technologies. For transient transfection assays, cells were set up in 6-well plates at a density of 2×10^5 cells/well. One day after plating, cells were transfected with 1 μg DNA/well using Lipofectamine reagent (Life Technologies) according to the manufacturer's instruction. After 3 h, the DNA–Lipofectamine complex was removed and the cells were replaced with fresh media. Two days after transfection, the cells were washed with phosphate-buffered saline and lysed in 200 μl reporter lysis buffer (Promega). Luciferase activities were measured using the Luciferase Assay System (Promega) and normalized with β-galactosidase activities to correct the transfection efficiency. All experiments were performed in triplicate. For the

treatment of RU486 or β-estradiol, media were switched with fresh one containing 1 μM RU486 or β-estradiol 24 h after transfection. Twenty-four hours after ligand treatment, the cells were harvested and luciferase activities were measured.

Results

Recombinant PurHG DBD constitutively binds PurR operator

The corepressor binding to the dimerized PurR is essential for DNA binding [6] (Fig. 1A). Based on the PurR structure, we designed possible dimeric PurR DBD (PurHG) (Fig. 1B) by fusing PurR DBD (residues 1–57) to GAL4 DD (residues 42–148). Recombinant GST fusion protein containing PurHG shows strong affinity to perfect palindrome of PurR operator in EMSA (Fig. 2C, lanes 7–11 and 13). The recombinant proteins without GAL4 DD (GST-Pur155) or a hinge helical element of PurR (GST-PurG) did not form the complex with PurR operator even at excess amount to 500 ng (Fig. 2C, lanes 1–6, 12, and 14). These results suggested that the hinge-helical region of PurR DBD and its dimerization by GAL4 DD are critical for DNA-binding activity of GST-PurHG and provided the direct evidence of the structural predictions that the proximating the hinge-helical region of PurR dimer is critical for DNA binding [10].

In order to identify DNA sequence specificity recognized by PurHG, we generated several mutant operator probes and tested its binding (Figs. 3A and B). The mutant probes mPuRE1, mPuRE2, and mPuRE3 were generated by the insertion of 'A,' 'AA,' or 'GC,' respectively, between the half sites of the perfect palindrome (Fig. 3A). Any insertion of nucleotides between the half sites of the palindrome completely abolished the complex formation with PurHG (Fig. 3B). The mPuRE4 has a 4 nucleotide mutation in the distal portion of the 16 bp palindrome. PurHG was able to bind to the mPuRE4 probe, although its affinity was much less than that of the perfect palindrome. But the mutations in central portion of the palindrome, mPuRE5, completely abolished DNA-binding activities (Fig. 3B). These results suggest that the nucleotide sequence at the center of the palindrome is critical for the binding of PurHG and it is the same characteristics of the natural PurHG [7,16].

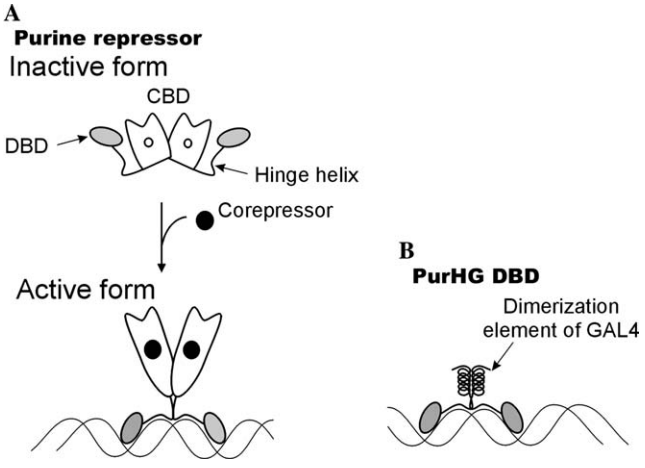

Fig. 1. A schematic diagram of the DNA-binding model of the *E. coli* PurR and recombinant PurHG DBD. (A) PurR composed of N-terminal DBD and C-terminal CBD. By binding of corepressor to CBP, PurR switches its structure to active conformation by changing the interaction between CBD and hinge helix of DBD. (B) The DD of GAL4 transcription stabilizes the hinge helix structure of PurR DBD to fit the minor groove of operator DNA without CBD.

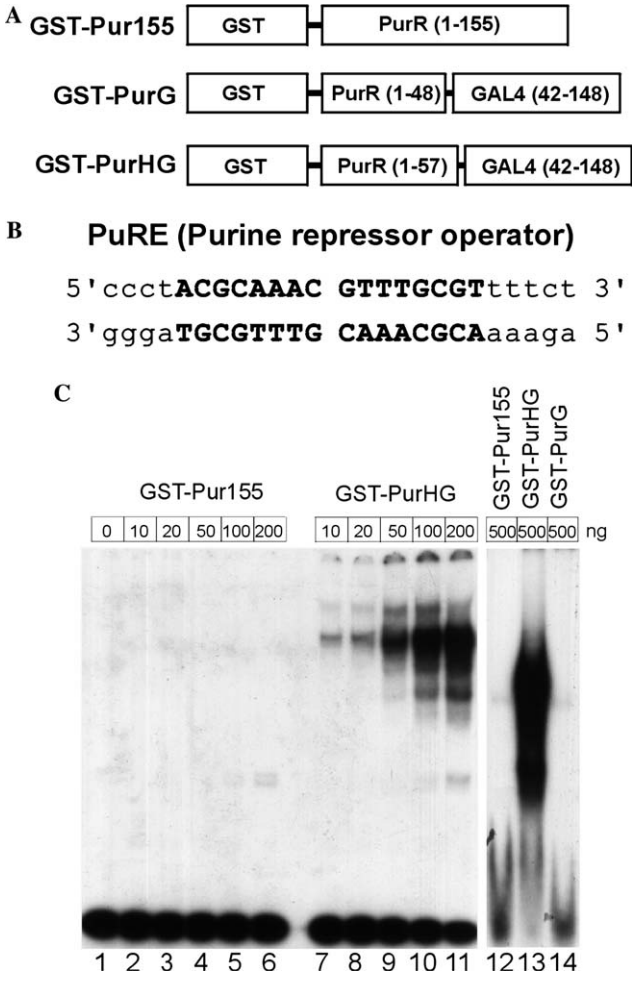

Fig. 2. Recombinant fusion protein of PurR DBD and Gal4 DD shows strong DNA binding. (A) The structures of the recombinant fusion proteins, GST-Pur155, GST-PurHG, and GST-PurG. (B) Symmetric PurR operator sequence (PuRE) probe used in EMSA. (C) DNA-binding characteristics of recombinant PurR proteins. EMSAs were performed, using the recombinant fusion proteins shown in A and PuRE probe. Indicated amounts of recombinant proteins, such as GST-Pur155 (lanes 1–6 and 12), GST-PurG (lane 14), and GST-PurHG (lanes 7–11 and 13), were incubated with ^{32}P-labeled PuRE probe and the protein–DNA complexes were separated on a 5% polyacrylamide gel, followed by exposure to X-ray film.

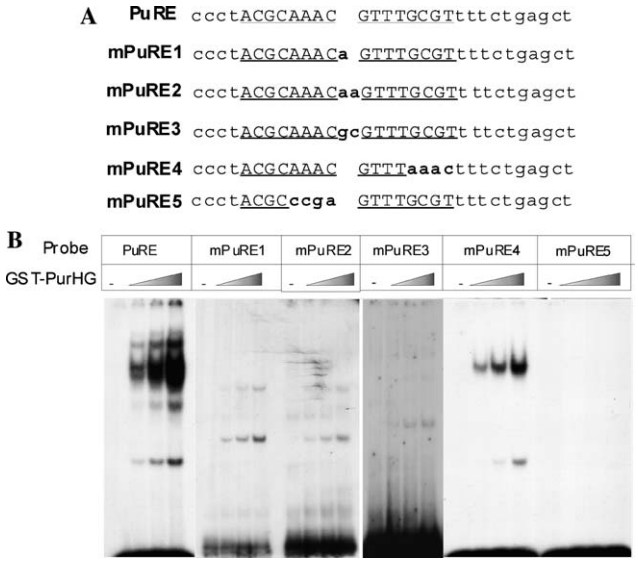

Fig. 3. DNA-binding characteristics of recombinant PurHG to mutated PuRE sequences. (A) Double-stranded mutant probes were made by the insertion of 'A' (mPuRE1), 'AA' (mPuRE2), or 'GC' (mPuRE3) between the half sites of PuRE or by changing four nucleotides of the distal (mPuRE4) or central portion (mPuRE5) of one half site. (B) EMSA of PurHG to mutated PuRE sequences. Each mutant probe was incubated with 0, 30, 60, or 120 ng recombinant GST-PurHG and EMSA was performed as described in Fig. 2C.

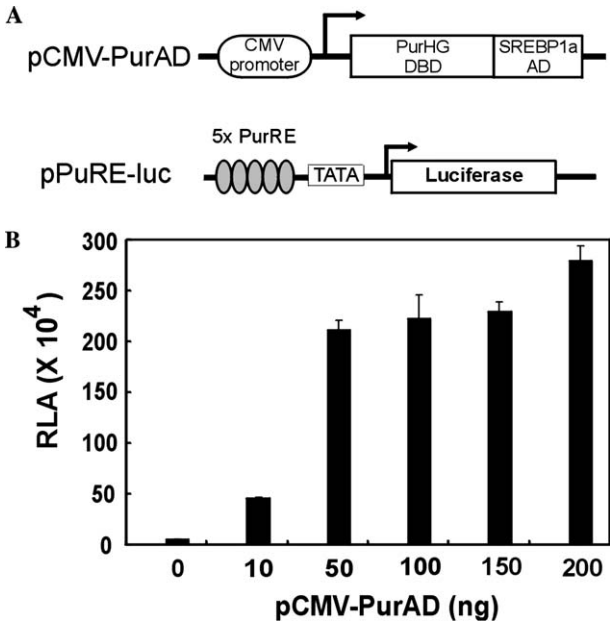

Fig. 4. PurHG can bind to PuRE sequence in eukaryotic cells. (A) The transcription activator, PurAD, recognizing the purine repressor operator, was constructed by fusion of the PurHG and activation domain (residues 1–79) of SREBP-1a. The reporter construct, pPuRE-luc, has five copies of PuRE at the upstream of the minimal ATP-citrate lyase promoter-luciferase construct as described in Materials and methods. (B) The NIH3T3 cells were transfected with pPuRE-Luc (150 ng), pCMV-βgal (20 ng), and indicated amounts of pCMV-PurAD. The cells were harvested 48 h after transfection and the luciferase activities were assayed. The luciferase activities shown in (B) were the values normalized by the β-galactosidase activities. The data represent means ± SD of three independent experiments performed in triplicate.

PurHG specifically binds to purine repressor operator in eukaryotic cells

In order to know whether the recombinant PurHG can act on the purine repressor operators in vivo, a CMV promoter-driven expression construct of a transcription factor which has the PurHG fused with the AD of SREBP-1a (residues 1–79) [11–13] was generated, and designated as pCMV-PurAD (Fig. 4A). A reporter plasmid pPuRE-luc has 5 copies of the PurR operator element (PuRE) at the upstream of modified ATP-citrate lyase minimal promoter [15], followed by luciferase gene (Fig. 4A). The PuRE used here is a 16 bp perfect palindromic consensus sequence (Fig. 2B) [7,9]. The luciferase activity was drastically increased by PurAD, even at low amount of 10 ng pCMV-PurAD, and reached a plateau of about 60-fold increase at 50 ng pCMV-PurAD (Fig. 4B). On the other hand, PurAD did not affect the expression of pGAL4RE-luc, which contains 5 copies of the GAL4-binding element instead of PuRE in pPuRE-luc (data not shown). These results suggest that the PurHG DBD can specifically bind to PuRE in eukaryotic cells.

Two artificial nuclear receptors, PurAPR and PurAER, containing LBD of progesterone receptor or estrogen receptor, respectively, were also generated and tested whether they activate reporter gene retaining PuRE in response to their ligands. PurAPR has LBD of the human progesterone receptor (645–891 amino acids), which was placed between the PurHG DBD and SREBP-1a AD. The LBD of the human estrogen receptor (279–554 amino acids) was similarly used in construction of PurAER. The progesterone LBD used here is a COOH-terminal deletion mutant, to which progesterone and other endogenous steroid hormones cannot bind but the progesterone antagonist RU486 still binds [17,18]. The PurAPR and PurAER markedly induced the luciferase activities of pPuRE-luc when their ligand, RU486 or β-estradiol, was added (Figs. 5B and C), while luciferase activities were not affected in the absence of the ligand. The responsiveness to the ligand reached maximum level at 50 ng of each construct expressing PurAPR or PurAER. The fold induction level of pPuRE-luc by PurAER in response to β-estradiol was much higher than that by PurAPR. These results show that PurHG DBD has full DNA-binding activity in eukaryotic cells and it could act as a transcription activator or a nuclear receptor by joining with an activation domain and a regulatory domain.

Dimerization by GAL4 dimerization element plays a critical role in DNA binding of the PurHG in eukaryotic cells

To confirm that dimerization of PurHG is indispensable for the DNA binding of PurHG in

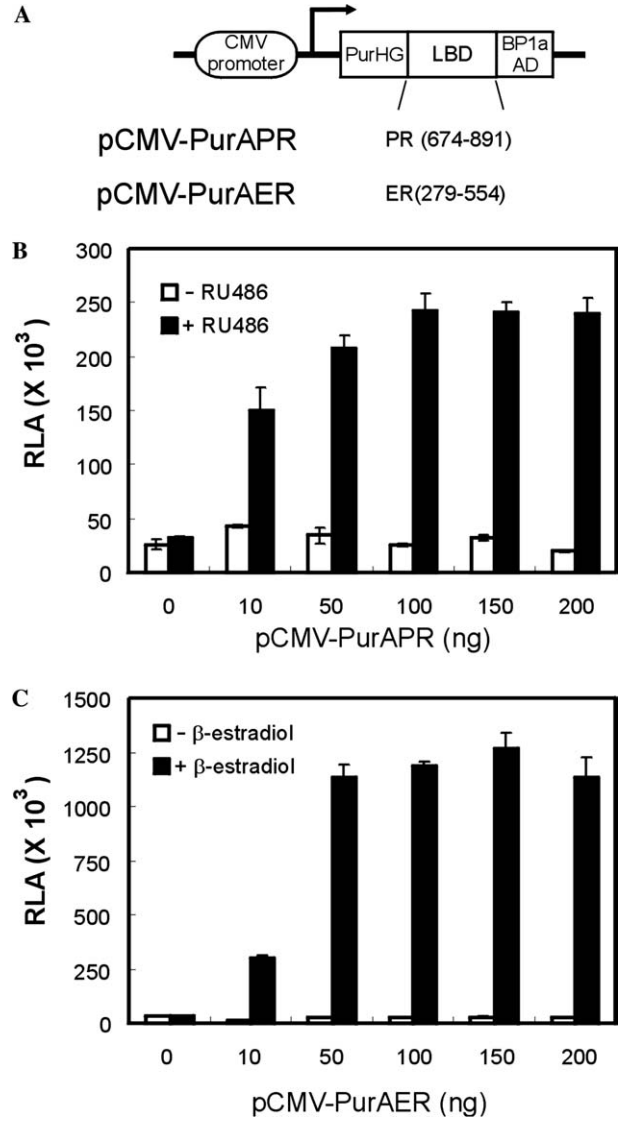

Fig. 5. Artificial nuclear receptors, PurAPR and PurAER, regulate the reporter gene expressions in response to the addition of a ligand. Artificial nuclear receptors, PurAPR and PurAER, were generated by insertion of the LBD of the progesterone receptor (674–891) or estrogen receptor (279–554) between the PurHG DBD and the SREBP-1a activation domain (A). The NIH3T3 cells were transfected with 150 ng pPuRE-Luc, 20 ng pCMV-βgal, and various amounts (0–200 ng) of pCMV-PurAPR (B) or pCMV-PurAER (C). After 24 h, RU486 or β-estadiol was added to the medium at the concentration of 1 μM and the cells were further incubated for 24 h. The reporter activities are shown as the relative luciferase activities normalized by the β-galactosidase activity. The data represents means ± SD of three independent experiments performed in triplicate.

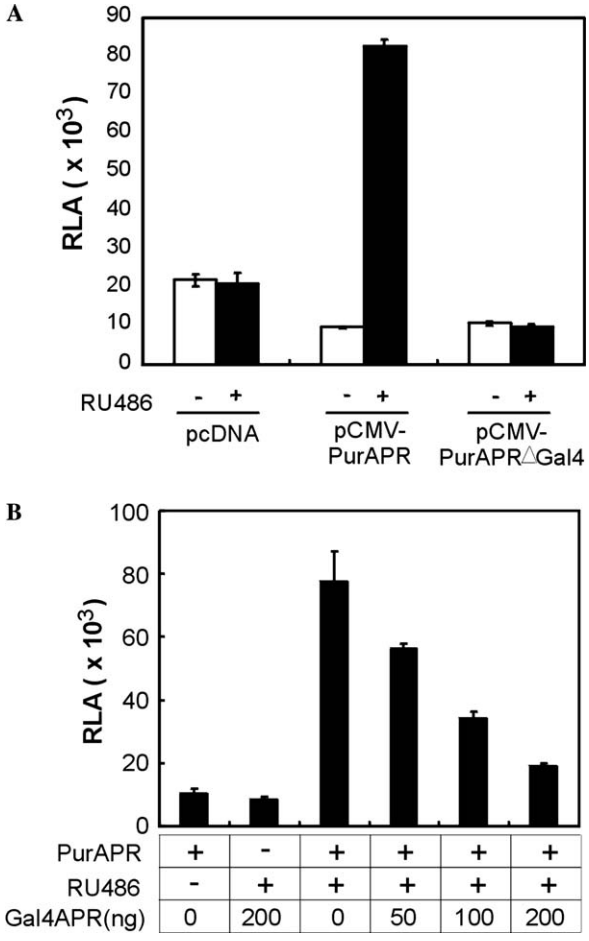

Fig. 6. Dimer formation by GAL4 DD is essential for the DNA binding of PurHG. NIH3T3 cells were transfected with pPuRE-Luc (150 ng), pCMV-βgal (20 ng) together with 200 ng of the expression vector (pCMV-PurAPR, pCMV-PurAPRΔGal4 or pCMV-GAL4APR). RU486-containing media wer replaced 24 h after transfection, and the cells were further incubated for 24 h. The cells were harvested and the luciferase and β-galactosidase activities were measured. The values of luciferase activities, normalized by β-galactosidase activities, are expressed as means ± SD of three independent experiments performed in triplicate.

eukaryotic cells, a dimerization mutant was generated from pCMV-PurAPR. The construct pCMV-PurAPRΔGal4 was generated by the deletion of the GAL4 dimerization domain from pCMV-PurAPR. The PurAPRΔGal4 did not induce the luciferase expression by RU486 at all (Fig. 6A). The dimerization of PurHG in eukaryotic cells was also confirmed by a competition experiment. The construct pCMV-Gal4APR has the same structure as pCMV-PurAPR except that it has the GAL4 DBD instead of the PurHG DBD. If the PurAPR acts as a dimer formed by the GAL4 DD, the overexpression of Gal4APR should inhibit the DNA binding of PurAPR by forming a heterodimer between Gal4APR and PurAPR, and thereby the RU486-responsive induction of luciferase expression of pPuRE-luc would decrease. The overexpression of Gal4APR inhibited luciferase expression induced by PurAPR and RU486 in a dose-dependent manner (Fig. 6B). This result suggests that the dimerization of PurHG, mediated by the GAL4 DD, occurs in eukaryotic cells and is critical for the DNA binding of PurHG.

Determination of minimal dimerization element by deletion of C-terminal region of GAL4 DD in PurAD and PurAPR

In the dimerized state of GAL4, two α-helices of DD are packed into a parallel coil–coil structure, like that found in the dimerization through the leucine zipper [19]. The region from amino acid residues 50 to 64 of the GAL4 dimerization element forms an amphipathic α-helix, and this region is dimerized only in the DNA-bound state [19,20]. Previously, it was shown that additional amino acid residues between 65 and 100 are required to maintain a dimer form in an unbound state. In order to determine the minimal requirement of the GAL4 dimerization element in biologically active artificial transcription factors, the COOH-terminus of GAL4 dimerization element of PurAD and PurAPR was deleted (Fig. 7). A deletion of 99–148 amino acid residues of the GAL4 dimerization element, designated as Gal98, did not affect the transcription activity in either forms of PurAD or PurAPR. However, a deletion of 76–148 residues (Gal75) significantly decreased the transcription activity of PurAD, but no significant loss of activity was detected in PurAPR. A deletion of 65–148 residues (Gal64) resulted in almost complete loss of activity in both forms of PurAD and PurAPR. These results suggest that the critical region for the GAL4 dimerization in PurAD context would be the amino acid residue 42–98 region, but in PurAPR, the 42–75 region seems to be enough for the dimerization. This is probably because the progesterone receptor LBD would complement the 76–98 region of GAL4 for dimerization.

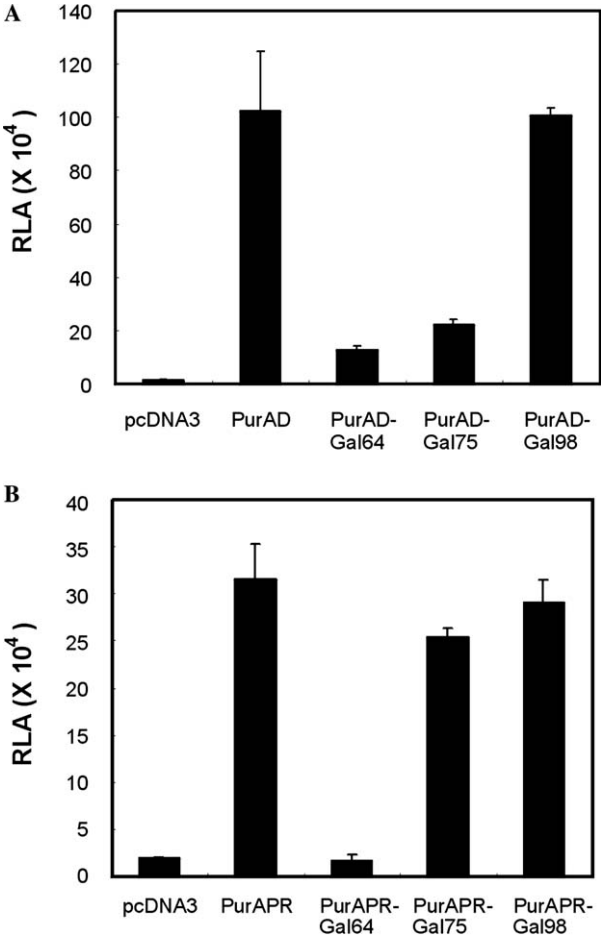

Fig. 7. The deletion of the COOH-terminus of the GAL4 DD differentially affects the biological activities in PurAD and PurAPR. The 3′ region of the GAL4 DD was deleted in constructs of pCMV-PurAD and pCMV-PurAPR. The resulting constructs, designated as Gal64, Gal75, and Gal98, contain the residues 42–64, 42–75, and 42–98 of GAL4 DD, respectively. The biological activities of each construct were determined in NIH3T3 cells by a transient transfection assay as described in Figs. 4 and 5. The values of luciferase activities, normalized by β-galactosidase activities, are expressed as means ± SD.

Discussion

Transcription activators consist of DBD and AD, and the recombination of DBDs and ADs from different transcription activators could produce the new artificial transcription activators. DBDs of most eukaryotic transcription factors bind to their binding consensus sequences of several endogenous genes and affect their gene expressions. Therefore, DBDs originated from prokaryote or yeast transcription factors, such as GAL4 [1,2] and LexA [4,21], are usually used in the development of the artificial transcription activators to control the expression of specific target gene. Recently, the 'mix and match' of designed or selected zinc fingers generated the novel DBDs with high affinity for desired target sequences [22–24]. These approaches have been developed to regulate the endogenous target gene expression [25,26]. The monomeric DBDs originated from zinc fingers can efficiently bind the DNA, but the multimerization by multiple binding elements in promoter is required for the transactivation of target gene.

The LacI family members have been a paradigm for understanding gene regulation and protein–DNA interactions. However, the DBDs originated from these members have not been used in construction of artificial transcription factors. *E. coli* PurR belongs to the LacI family of transcriptional regulators. Most LacI family members have a high affinity to the operator sequences in unliganded states. However, PurR needs a corepressor to bind its operator sequences. Proper orientation of hinge helix for DNA binding of LacI family is mediated by cofactor-binding domain. The critical role of cofactor-binding domain in DNA binding hindered the DBDs of Lac family members from their applications to generation of artificial transcription factors. Recently, Kalodimos et al. [27] showed that the dimerization of LacI DBD by disulfide bond in vitro creates the strong DNA-binding activity without the help of cofactor-binding domain. In present study, we generated a novel

dimeric PurR DBD by fusing the COOH-terminus of PurR DBD to GAL4 DD. Resulting PurHG showed strong DNA-binding activity both in vitro and in vivo. The dimerization and hinge-helical region of PurR DBD were proved to be critical for DNA binding, by showing that the deletion of GAL4 DD or hinge helix from PurHG completely abolished the DNA binding. Polypeptide comprising the first 147 amino acids of GAL4 binds DNA as a dimer in vitro [20]. GAL4 DD consists of three short dimerization elements at position 55–66, 78–84, and 87–97, forming parallel coiled-coil dimeric structure [28]. The residues 42–98 of GAL4 DD is the minimal region of GAL4 DD required for functional PurHG in context of PurAD, transcriptional activator. This result corresponds to the fact that GAL4 DBD, encompassing residues 1–66, is not dimerized in free protein solution, until it forms the complex with DNA and the extension to residue 100 is required to maintain a stable dimer in unbound state [19]. However, in form of PurAPR, artificial nuclear factor, the residues 42–75 of GAL4 DD are sufficient for DNA binding of PurHG. It is interesting that the first helical region (residues 42–66) of GAL4 DD by itself did not generate the DNA binding and the loop region of residues 67–75 should be included for PurAPR activity. Previous data that progesterone receptor LBD itself is involved in dimerization [29,30] might explain that progesterone receptor LBD can be substituted for the second and third helices of GAL4 DD.

The perfect palindrome of the 16 bp PurR operator showed the strongest affinity to the designed DBD of PurHG. Any insertion of nucleotides between half sites completely abolished PurHG binding. However, the mutations at the distal 4 bp in the half site were able to form a complex with PurHG, even if the affinity was markedly decreased. These results indicated that PurHG recognizes the same DNA sequence as the wild PurR, and the central portion of the palindrome is important for its recognition.

In summary, we have created a novel DBD using the *E. coli* purine repressor DBD, which could be used in constructing artificial transactivators or artificial nuclear receptors. This study provides the possibility that at least 21 members of the *E. coli* LacI family, recognizing different specific DNA sequences, might be reconstructed into constitutive DBDs, being applicable to generation of artificial transcription activators or nuclear receptors.

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