Development of a novel DNA-binding domain derived from *Escherichia coli lac* repressor and its application to artificial eukaryotic transcription factors

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Development of a novel DNA-binding domain derived from *Escherichia coli lac* repressor and its application to artificial eukaryotic transcription factors

Directed by Professor Kyung-Sup Kim

A master's thesis submitted to the Department of Medical Science, the Graduate School of Yonsei University in partial fulfillment of the requirements for the degree of master of Medical Science

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August 2003

This certifies that the master's thesis of
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The Graduate School
Yonsei University
August 2003

Acknowledgements

모든 것을 가능케 하신 하나님께 모든 영광을 돌립니다.

이 논문이 완성되기까지 이끌어 주시고 아낌없는 지도를 베풀어 주신 은사 김경섭 선생님께 진심으로 감사드리며, 부족한 논문을 심사하여 주신 안용호 선생님과 김종선 선생님께도 감사를 드립니다. 또한 따뜻한 관심을 가져 주셨던 오상환 선생님, 허만욱 선생님, 김건홍 선생님, 김재우 선생님께 깊은 감사를 드립니다.

제가 졸업을 할 수 있었던 것은 많은 사람에게 받은 사랑의 빚이라고 생각됩니다. 실험실에서 늘 웃음 지을 수 있도록 희노애락을 함께 해준 정안이, 성진이, 정민이, 재은이, 소영언니, 사라언니, 우석오빠, 동기쌤. 그리고 여러 가지로 많은 도움을 주신 생화학 교실의 모든 분들. 너무 고맙습니다. 특히 실험의 기초부터 세심하게 다져주시고 많은 배려를 해주셨던 박상규 선생님께 고마운 마음을 전합니다.

저의 삶의 기도와 사랑의 후원자이신 부모님과 언니, 동생에게 이 논문을 드립니다.

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Development of a novel DNA-binding domain derived from *Escherichia coli lac* repressor and its application to artificial eukaryotic transcription factors

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(Directed by Associate Professor **Kyung-Sup Kim**)

Novel DNA-binding domain (DBD), LacHG, was designed by joining the lac repressor DBD to GAL4 dimerization domain (DD), which could constitutively bind to lac operator. To assay the DNA-binding activities of LacHG, it was fused to the activation domain (AD) of SREBP-1a, resulting in artificial transcription activator, LacAD. The deletion of linker region (42-49 a. a.) of GAL4 DD, significantly enhanced the transcription activities of LacAD, while the linker deletion in PurHG, derived from purine repressor

(PurR), decreased the transcription activities of PurAD. In reporter construct, the symmetric lac operator sequence [SymL(-1)] responded to LacAD much more efficiently than wild lac operator sequence.

The LacHZ was generated by replacement of GAL4 DD in LacHG with GCN4 leucine zipper, which is well known as dimer forming motif. The LacHZ-AD activates the receptor expression as much as LacAD. The addition of nuclear localization signal (NLS) of SV 40 large T antigen to LacHZ-AD did not increase transcription activities.

We made artificial nuclear receptor by insertion of ligand-binding domain (LBD) of progesterone receptor (PR, 645-891 a. a.) or estrogen receptor (ER, 279-554 a. a.) between DBD and AD for ligand-dependent activation of reporter gene. The mLacAPR, containing PR LBD, induced the reporter expression to about 6 folds in respond to RU486, even if this was much less than 12 fold induction in PurAPR containing PurHG instead of LacHG. The mLacAER, containing ER LBD, markedly increased the

transcription of luciferase gene in respond to β-estradiol to about 63 folds comparable to PurAER.

In present study, lac repressor DBD was first modified to constitutive DBD which specifically bind to lac operator sequence. Transcription factors, containing chimeric DBD derived lac repressor DBD, works well in eukaryotes as in forms of transcription activator or nuclear receptor.

Key words: lac repressor, DBD(DNA binding domain), GAL4 AD(activation domain), LBD(ligand binding domain),

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I. Introduction

Regulation of gene expression in eukaryotic cells occurs primarily at three distinct levels, such as transcription, RNA processing, and translation level. Control of transcriptional level is the most important mechanism by which eukaryotic cells selectively synthesize proteins at any given time. Transcription control is governed by the actions of a large number of transcription factors. Transcription factors typically contain at least two domains: a DNA-binding domain (DBD) that binds to a specific sequence of

base pairs in the DNA, and an activation domain (AD) that activates transcription by interacting with other proteins. Because those domains generally work independently, artificial transcription factors can be generated by swapping the DBDs between transcription factors. Artificial transcription factors working in animal cells were constructed for regulating the expression of target reporter gene without affecting the endogenous gene expression.[1-2] These purposes limit the repertoire of DBDs to those derived from transcription factors of , such as LexA[3-4] and GAL4[2,5] or designed zinc finger DBDs[6].

Lac repressor is 155 kDa tetrameric protein that binds to operator sites of target genes[7]. Lac repressor is composed of three structural domains, which are the NH_2-terminal DBD (residues 1-62), the core domain (residues 63-340) and the COOH-terminal domain (residues 341-357)[8-10]. The NH_2-terminal DBD consists of the helix-turn-helix motif and hinge helix, which is involved in the DNA-binding when the inducers, such as allolactose and IPTG, do not bind to

the core domain[11]. The inducer binding to core domain triggers the conformational changes, and altered the orientation of NH_2-terminal DBD, which, in turn, displaces the hinge helix from the minor groove of the operator[11]. The COOH-terminal helical structure stabilizes the tetrameric structure by the dimerization of lac repressor dimer[12]. The lac operon have been a paradigm for understanding gene regulation and protein-DNA interactions. This regulatory system is widely used as a tool of recombinant protein expression in prokaryotes. Recently it was applied to a inducible gene expression system in transgenic animal[13]. Despite a lot of structural and biological information about lac repressor, its DBD have not been used in generation of artificial transcription factor working in animal cells. Yeon had created the noble DBD, designated as PurHG, by fusion of purine repressor (PurR) DBD with GAL4 dimerization domain (DD)[14]. PurHG constitutively binds to PurR operator regardless of the presence of corepressor. PurR and lac repressor belong to LacI family, sharing the high sequence homology. The

sequence identity is greatest in NH_2-terminal DBD[15-17].

Here, we designed the chimeric DBD, LacHG, by fusing lac repressor DBD to GAL4 DD. We also generated LacHG-containing transcription factors and tried to improve their transcriptional activities through several modifications in chimeric DBD and reporter constructs. This study might provide the insights in generating constitutive DBDs from large members of LacI family and the possibility of designed DBDs targeted to desired specific sequence by recombination or substitution of motifs between DBDs.

II. Materials and Methods

1. Construction of recombinant DNA-binding domain

A. Construction of DNA-binding domain

The lac repressor DBD (residues 1-62) was amplified from pGEX4T3 vector (Amersham Pharmacia, Buckinghamshire, UK) using LacDBD-F (5'-CTGAGATCTGCCACCATGAAACCAGTAACGTTATACGAT-3') and Lac DBD-B (5'CCTTTTGGTTTTGGGCAACGACTGTTTGCCCGC-3'). And the coding region for GAL4 dimerization element (residues 42-148) was amplified from pFA2-Elk1 (Stratagene, La Jolla, CA, USA) using GAL4 DD-F (5'-AAACAGTCGTTGCCCAAAACCAACAGGTCTCC-3') and GAL4 DD-B (5'TGAAAAAGTGGATCCGGGCGATACAGT-3'). To generate LacHG, it was amplified from mixture template of amplified LacDBD and GAL4 dimerization element using LacDBD-F and GAL4 DD-B. The LacHG was then ligased with pcDNA3.0 (Invitrogen, Carlsbad, CA, USA) using the BglII and BamHI restriction sites incorporated in the PCR primers(1,4). For

construction of the mLacHG, linker region of the GAL4 dimerization element was deleted by amplification using mGAL4 DD-F (5'-CAGCGGAGACCTTT TGGTGGATCCGTTAACCTTCAGGCT-3') and GAL4 DD-B. To generate the LacHZ, GAL4 dimerization element was replaced by amplified the GCN4 Leucine zipper dimerization element using Leuzip-F (5'-CAGTCGTTGATGA AACAACTTGAAGACAAGGTTGAAGAATTGCTTTCGAAAAATTATCA CTTGGAAA-3') and Leuzip-B (5'- CTAGGATCCCGTTCGCCAACTAATT TCTTTAATCTGGCAACCTCATTTTCCAAGTGATAATTTTTC-3'). The pCMV-LacAD constructs as transcription activator was produced by inserting activation domain of SREBP-1a into the BamHI site of pCMV-LacHG. Others transcription activators such as mLacAD and LacHZ-AD were designed by same procedures as in pCMV-LacAD. And the LacHZ(NLS) was produced by linking DNA segments encoding the NLS between LacHZ and AD (Fig.1).

B. Construction of artificial nuclear receptor

The artificial nuclear receptor constructs containing PR LBD or ER LBD

were produced by inserting the BamHI and EcoRI fragment from pCMV-GAL4ANR into the BamHI and EcoRI site of pCMV-LacHG, pCMV-mLacHG, pCMV-LacHZ and pCMV-LacHZ(NLS) (Fig.1).

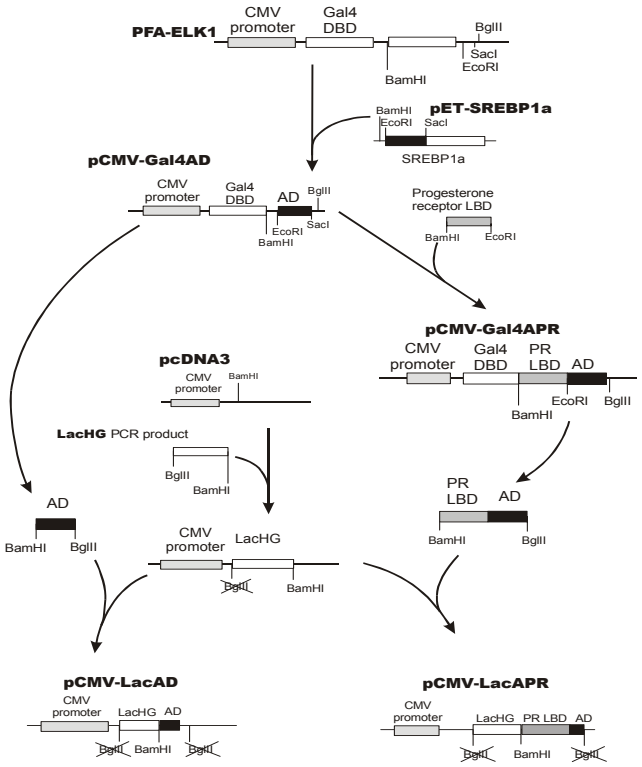

Fig1. The construction of plasmid vectors expressing artificial transcription factors. The procedures, cloning the expression vectors, were described in Materials and Methods.

C. Construction of reporter plasmid

The reporter constructs of pLaRE and pSymL(-1) were generated by inserting 3 repeats of LaRE or SymL(-1) sequences into SacI site upstream of the modified rat ATP citrate-lyase minimal promoter (-60 to +67), which has 'TATAAA' sequence at 30/-25 site instead of original 'GACAAA' sequence, followed by luciferase gene[18].

2. Cell culture and Transfection

NIH3T3 cell line was maintained in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% fetal bovine serum, 100 unit/ml penicillin G sodium, 100 μg/ml Streptomycin sulfate in 5% CO_2 at 37°c. All cell culture materials were purchased from Invitrogen (Carlsbad, CA, USA) Cells were set up in 6-well plates at a density of 1.5x 10 5cells/well. In next day, cells were transfected with 1μg of DNA/well Lipofectamine Reagent (Invitrogen, Carlsbad, CA, USA, Inc.) according to the manufacturer's instruction. After 3 h, DNA- Lipofectamine complex was removed and the

cells were replaced with fresh media. On day 3, cells were washed with phosphate-buffered saline and lysed in 200$\mu\ell$ of reporter lysis buffer (Promega, Medison, WI, USA). Luciferase activities were measured using the Luciferase assay System (Promega, Medison, WI, USA) and normalized with β-galactosidase activities to correct the transfection efficiency. All experiments were done in triplicate and were repeated at least once.

For the RU486 (Sigma, St. Louis, MO, USA) treatment experiments, the medium as changed to 1 μM RU486 containg media at 24h after tranfection (day 2). On day 3, cells were harvested and luciferase activities were measured. The β-estadiol (Sigma, St.Louis, MO, USA) was treated as same.

III. Results

1. Construction of chimeric DBD and artificial transcription factors

We designed a novel DBD originated from lac repressor like the instance of PurHG, which is the reconstructed DBD using *E. coli* PurR DBD[14]. To generate dimeric hinge-helical structure of lac repressor DBD to fit the minor groove of operator, we introduce GAL4 DD (residues 42-148)[19] at COOH-terminus of lac repressor DBD (residues 1-62)[11] (Fig.2A). To determinate recombinant DBD activity in eukaryotic cells, we generated transcription activator, designated as LacAD, by connecting it to SREBP-1a AD (residues 1-79)[20-21] to the COOH terminus of LacHG. For the controllable expression system, we also designed artificial nuclear factors, designated as LacAPR and LacAER, which contain LBD of progesterone receptor (PR, residues 645-891) or estrogen receptor (ER, residues 279-554) between LacHG and SREBP-1a AD. The PR LBD is a mutated form by the deletion of the COOH-terminus, which progesterone and other endogenous steroid

hormones cannot to bind but the progesterone antagonist RU486 can still bind[23] (Fig. 2A).

The luciferase reporter plasmids, such as pLaRE-luc and pSymL(-1)-luc, have 3 copies of wild-type repressor operator element (LaRE) or 4 copies of symmetric lac operator element [SymL(-1)] at the upstream of minimal promoter (Fig. 2B).

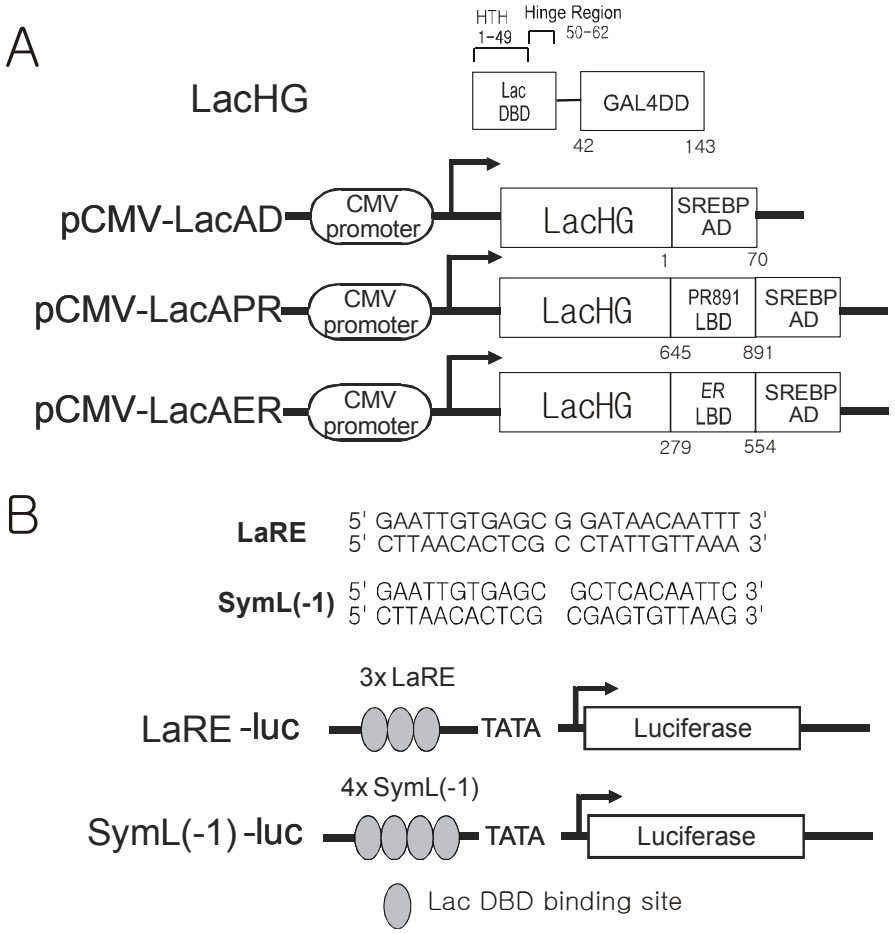

Fig2. Schematic representation of transcription activator, artificial nuclear receptor and reporter constructs.

(A) Structures of transcription activators. The LacHG was generated by fusing lac DBD (1-62 a. a.) to GAL4 DD (42-143 a. a.). The artificial transcription factors were constructed by recombination of LacHG, AD of SREBP-1a (1-79 a. a.), and LBDs of PR (645-891 a. a.) or ER (279-554 a. a.). (B) The luciferase reporter plasmids. Reporter constructs were prepared by insertion of 3 copy wild-type lac operator (pLaRE-luc) or 4 copy SymL(-1) [pSymL(-1)-luc] upstream of ATP-citrate lyase minimal promoter[18]. LaRE (23bp) is the wild lac operator sequence and SymL(-1) (22bp) are synthetic symmetric operator sequence.

2. Measurement of DNA-binding activities of chimeric DBD in eukaryotic cells.

In order to determine the DNA-binding activity of LacHG, we measure the transactivation of reporter gene by LacAD, using transient transfection assay in NIH3T3 cells. The overexpression of LacAD increased the luciferase expression from pLaRE-luc construct by 9 folds. Transactivation by LacAD was shown 8 fold higher in pSymL(-1) than in pLaRE-luc, even though the basal levels of pSymL(-1)-luc is about 3 fold higher than pLaRE-luc (Fig.3A). This result corresponded to previous report that SymL(-1), 22bp symmetric synthetic operator, shows tightest binding operator[24].

We designed the artificial nuclear receptors, which can induce the gene expression when the ligand is supplied. The LacAPR, containing PR LBD, did not affect the basal transcription of pLaRE-luc, but slightly increase the luciferase gene expression of pSymL(-1)-luc in absence of RU486. The responsiveness to RU486 of LacAPR was absent in pLaRE-luc, and very weak

with 1.5 fold increase in pSymL(-1)-luc (Fig.3B). The ER LBD, used in construction of ligand-dependent transcription factor generally includes the E and F domains[6,25,26], but in present study, we used the only E domian for constuction of pCMV-LacAER. It was reported that the ER LBD without F domain could bound to estrogen.[14] When the LacAER was overexpressed, estrogen induced luciferase activities 10 folds in pLaRE and more drastically in pSymL(-1) by 19 folds. When comparing the absolute values, the estrogen-induced luciferase activities in pSymL(-1) revealed 14 folds than those in pLaRE (Fig.3B).

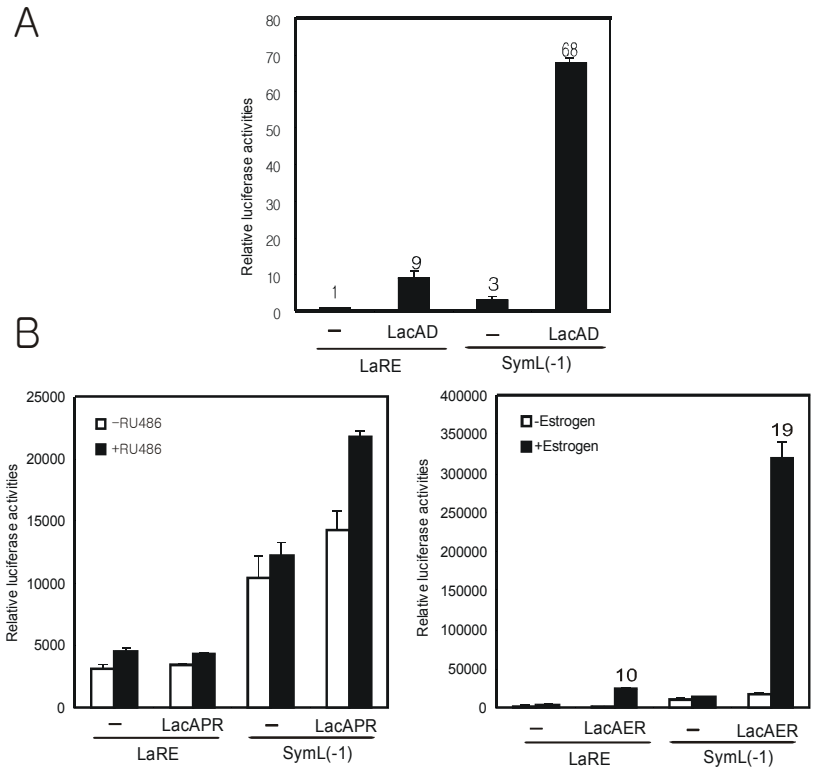

Fig. 3 Transactivation of pLaRE-luc and pSymL(-1)-luc by artificial transcription factors containing LacHG.

(A) Transactivation by LacAD. (B) Transactivation by artificial nuclear receptors, LacAPR and LacAER. NIH3T3 cells were transfected with 50 ng of pCMV-β-gal(50ng), 150 ng of reporter construct (pLaRE-Luc or pSymL(-1)) and 150 ng of the expression vector (pCMV-LacAD, pCMV-LacAPR or pCMV-LacAER). In cases of ligand-dependent activation, the ligand, such as 1 μM RU486 or β-estradiol, was treated 24 h after transfection. Luciferase and β-galactosidase activities were measured 48 h after transfection and luciferease activities were normalized by β-galactosidase acticities.

3. The modifications of chimeric DBD.

To determine whether GAL4 DD is required for DNA binding of LacHG, LacH-AD, not containing GAL4 DD, were constructed and its transactivation activities were checked. LacH-AD did not have transactivation activity. This result suggested that the dimerization of lac repressor DBD is absolutely needed for DNA-binding. The orientation of dimerized hinge helixes is important for DNA-binding affinity and this fact suggests the possibility that the distance between hinge helix and GAL4 DD might affect the DNA-binding affinity. To confirm this possibility, the linker region (42-49 a.a) of the GAL4 dimerization element was removed (Fig.4A). The resulting construct, mLacHG, drastically increased the luciferase expression from pSymL(-1) by 45 folds (Fig. 4 B). Next, we replaced the GAL4DD of LacHG with GCN4 leucine zipper. GCN4 leucine zipper is known well as dimer-forming motif with a parallel coiled-coil element[27]. Resulting construct, LacHZ-AD, increased the expression of luciferase gene 23 folds with similar level to the

transactivation by LacAD. To observe whether nuclear localization signal (NLS) of SV 40 large T antigen helps the movement of LacHZ-AD protein into the nucleus and increases its activity, NLS was inserted between LacHZ DBD and SREBP-1a AD. However, resulting LacHZ(NLS)-AD did not affected the transactivation activities of LacHZ-AD (Fig.4 A,B). To determine the optimal dose of mLacAD for maximal tranactivation, we changed the amounts of pCMV-mLacAD in transient transfection assay. The luciferase activities were drastically increased even by 10ng of pCMV-mLacAD, reached the maximum by 60 folds at 100ng, and were slightly decreased at the amounts above 100ng (Fig.5). The above results indicated that mLacAD showed highest transactivation activities at the dose of 100ng of pCMV-mLacAD. Next, we compared the effect of GAL4-linker deletion in chimeric DBDs originated from lac repressor and PurR DBD. In contrast to the induction by the deletion of linker region in LacAD, the transactivation activities were decreased by linker deletion in PurAD (Fig. 6). Although we cannot precisely

explain this difference between LacAD and PurAD, the orientations of hinge helixes for optimal DNA-binding might be somehow different between lac repressor family members and require the different dimeric structure.

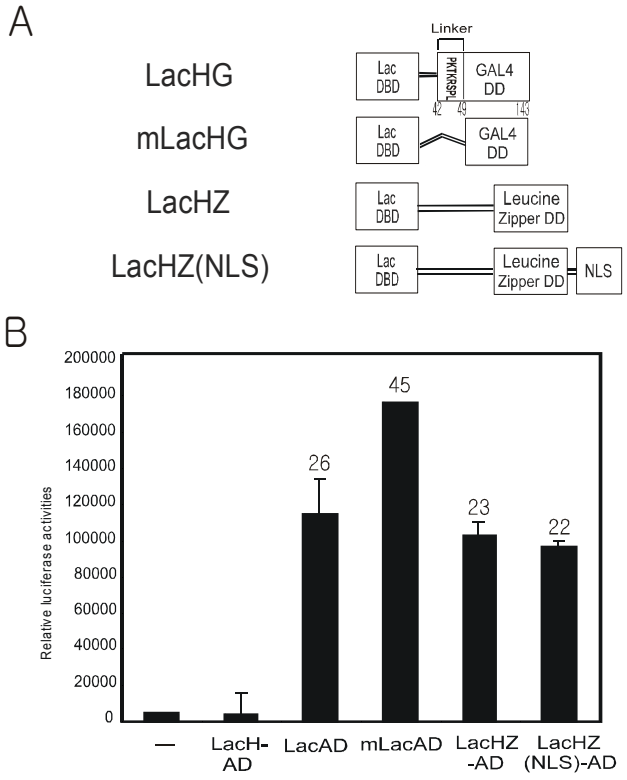

Fig. 4. Variants of chimeric DBD derived from lac repressor and their DNA binding activities in eukaryotic cells.

(A) Chimeric DBDs generated by fusion of lac repressor DBD and various dimerization elements. Lac repressor DBD (1-62 a.a.) was connected several dimerization domains, such as residues 42-143 or residues 49-143 of GAL4 DD, or GCN4 leucine zipper (32 a.a.), resulting in LacHG, mLacHG, and LacHZ, respectively. LacHZ(NLS) was generated by joining the nuclear localization signal of SV40 large T antigen to COOH-terminus of LacHZ. (B) Transactivation by artificical transactivators containing various chimeric DBDs. Transient transfection assays were performed as described in Fig. 3.

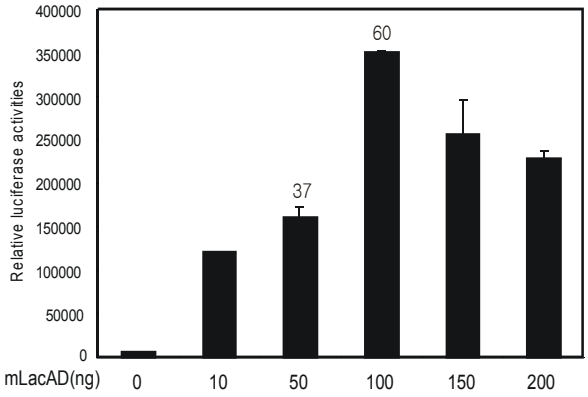

Fig5. Determination of optimal dose of pCMV-mLacAD.

The NIH3T3 cells were transfected with 150ng of pSymL(-1), 50ng pCMV-β-gal and various amounts(0 to 200ng) of pCMV-mLacAD. Luciferase activities were assay 48 h after transfection and normalized by β-galactosidase activities.

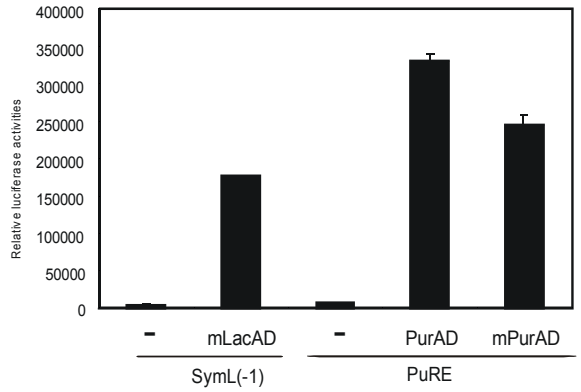

Fig6. The effects by deletion of GAL4-linker region (residues 42-48) on transactivation activities of LacAD and PurAD.

The NIH3T3 cells were transfected with 150ng of pSymL(-1), 50ng pCMV-β-gal and 100 ng of respective expression vectors. Luciferase activities were assay 48 h after transfection and normalized by β-galactosidase activities.

4. Ligand-dependent transactivation by artificial nuclear receptor

We made artificial nuclear receptors, using chimeric DBDs which were generated by fusion of lac DBD to several DDs. Among the artificial nuclear receptors containing PR LBD, only mLacAPR, containing mLacHG, induced the luciferase activities 6 folds (Fig.7A). In contrast to lac repressor DBD-containing nuclear receptors, PurAPR drastically activated the transcription 12 folds by RU486 and the deletion of GAL4 linker region (mPurAPR) did not affect its transactivation activities (Fig.7A). In cases of ER LBD-bearing nuclear receptors, LacAER and LacHZ-AER markedly increased the luciferase activities about 10 to 12 folds, and especially, the induction by mLacAER in response to RU486 reached 63 folds, which is much higher than by PurAER or mPurAER (Fig.7B). The above results showed that ER LBD-bearing nuclear receptors revealed much more potent responsiveness to ligand than PR LBD-bearing ones in chimeric lac repressor DBD-based system.

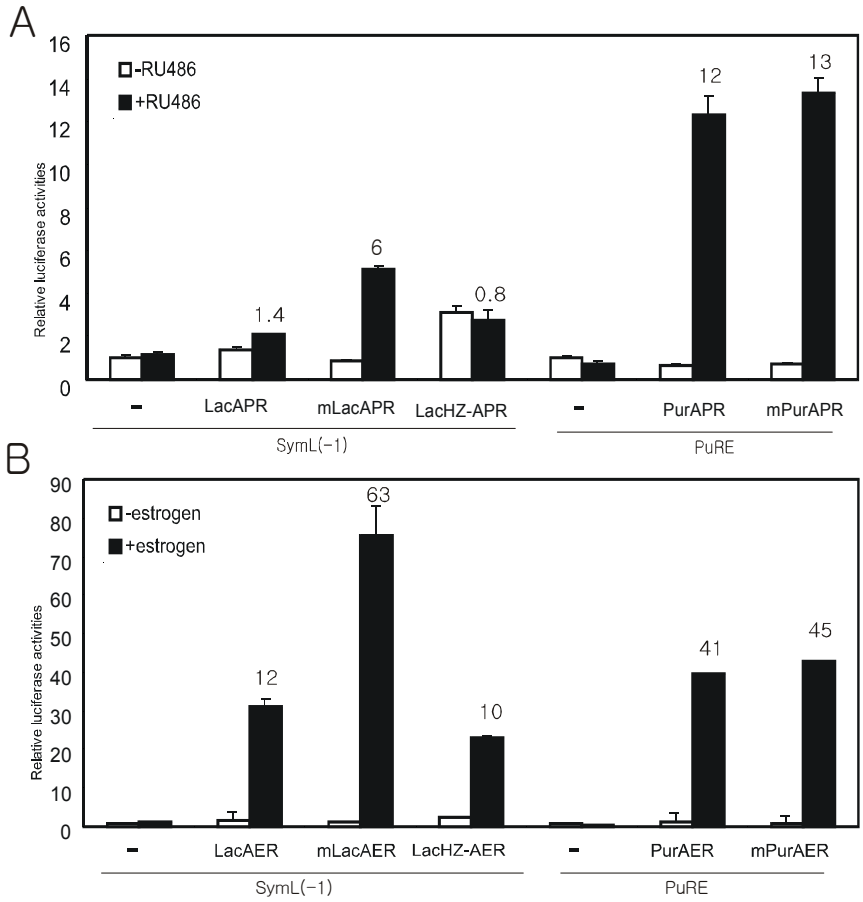

Fig7. The ligand-dependent transactivation by artificial nuclear receptor containing chimeric DBDs derived from lac repressor DBD.

(A) and (B) transactivation by PR LBD or ER LBD-bearing nuclear receptors.

The artificial nuclear receptors were generated by insertion of PR LBD (674-891 a. a.) or ER LBD (279-554 a. a.) between DD and AD. The each constructs (100ng) were transfected into NIH3T3 cells with 150ng of pSymL(-1), 50ng pCMV-β-gal. The cells were treated with containing 1 μM of RU486 or β-estradiol 24 h after transfection and luciferase activities was measured 48h after transfection. The relative luciferase activities were shown as values normalized by β-galactosidase activities.

IV. Discussion

Most transcription activators consist of DBD and AD. The recombination of DBDs originated from prokaryote/yeast and AD from different transcription activators could develop the new artificial transcription activators. And it can be used invaluable tools for basic and applied researches.

Despite of the large repertoire of LacI family, their DBDs have not been used in construction of artificial transcription factors, because DNA binding activity of most LacI family members is affected by existence of inducer or corepressor[8, 10, 11, 28]. The residues 1-49 of lac repressor form the 'headpiece', a three-helix bundle that binds to the major groove of the operator[11]. *E.coli* lac repressor is tetramer as a dimer of dimer, where each dimer can bind lac operator[9]. The headpieces are connected by a hinge helix (residues 50-62) of which the dimeric structure is known to play a crucial role in DNA-binding[11, 29]. The inducer binding to the core domain alters the interaction between core domain and DBD, thereby dissociating the hinge-helix from minor groove of

operator. In previous study, DBD of purine repressor was designed to constitutive DBD binding to purine repressor operator with high affinity regardless of the presence of corepressors and named as PurHG[14]. In present study, we have created the new DBD from lac repressor DBD by introducing the dimeric structure to stabilize the hinge helix. GAL4 is transcription activator to regulate the genes involved in galactose and melibiose metabolism in Saccharomyces cerevisiae[30]. The distinct functional domains were reported, such as DNA-binding domain, transactivation domain, and dimerization domain[31-33]. GAL4 DD consists of three short dimerization elements at position 55-66, 78-84 and 87-97, forming parallel coiled-coil dimeric structure[34]. GAL4 DBD, encompassing residues 1 to 66, is not dimerized in free protein solution, until it forms the complex with DNA. The extension to residue 100 is previously reported to be required to maintain a stable dimer in unbound state[19]. The GAL4 DD, encompassing the residue 42 to 143, was reported to efficiently dimerize the purine repressor DBD, resulting in active

DBD, PurHG[14]. In present study, we constructed LacHG by joining GAL4 DD (residues 42 to 143) and lac repressor DBD (residues 1 to 62). However, LacHG showed low transcriptional activities in context of transactivator (LacAD) or nuclear receptors (LacAPR or LacAER). Since the orientation of hinge helix plays a critical role in DNA-binding, the mutation on LacHG was made with the deletion of linker region of GAL4 DD (residue 42 to 49). This mutation drastically increased the transcriptional activities of both transcription activator and nuclear receptors, while this mutation on PurHG of artifitial transcription factors, such as PurAD, PurAPR or PurAER, decreased or did not affected their transcriptonal activities. These facts suggested that the orientations of the hinge helix, fitting for the DNA-binding, might be different between lac family members and require different dimeric structures.

Another most well known motifs for dimerization is the leucine zipper. The leucine-rich helices forms a dimer by a hydrophobic interaction with each other[35]. Yeast transcription factor, GCN4, is the one of the leucine-zipper

transcription factors, of which the COOH-terminal 30 residues, containing leucine repeat, forms the dimer by itself[27, 36]. GCN4 leucine zipper motif was used for the dimerization of lac repressor DBD. In context of transcription activator (LacHZ-AD), transcriptional activities were not high to a similar level to those of LacAD. LacHZ-APR increase the transcription in ligand -independent manner, suggesting possibilities that the retention of LacHZ-APR in the cytosol in absence of ligand might be prohibited and activate the reporter gene expression. Ligand-dependent transactivations of LacHZ-AER were very low in comparison to those of artificial nuclear receptors containing mLacHG. Animal cells have many kinds of transcrition factors containing leucine zipper motif, such as C/EBP, Jun, Fos, Myc, and so on, which can form heterodimer. This fact provides the possibility that the artificial transcription factors using LacHZ DBD could not efficiently activate the reporter gene due to heterodimer formation with endogenous transcription factors.

In summary, we have designed the artificial transactivator or artificial

nuclear receptors by using *E.coli* lac repressor DBD. The mLacHG of chimeric DBDs have the strongest affinity to the lac operator sequence regardless of inducer in eukaryotic cells. This study showed that a lot of DBDs of lac family members could be used in reconstruction of constitutive DBDs. Moreover, like zinc finger motif DBD, the simple and short structure of lac family DBD might provide possible tools to target desired sequence by modification and substitution between these DBDs in future.

Ⅴ. Conclusion

In this study, we created the new DBD by generating the chimeric protein of lac repressor DBD and GAL4 DD. Deletion of GAL4 linker region from LacHG DBD enhanced the transcriptional activities of artificial transcription factors. Artificial nuclear receptor, LacAPR, containing PR LBD responded to RU486 to less extent than PurAPR, whereas LacAER containing ER LBD highly efficiently induce the transcription of reporter gene in respond to β-estradiol.

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초 록 (In Korean)

Escherichia coli lac repressor에서 유래된 새로운 DNA-binding domain의 개발과 이를 이용한 유핵 세포에서의 전사조절

E.coli lac repressor DNA-binding domain (DBD)와 yeast의 GAL4 dimerization domain (DD)을 결합시켜 corepressor와 무관하게 항시 lac operator에 결합능력이 있는 새로운 DBD인 LacHG를 만들었다. 이 DBD에 SREBP-1a activation domain(AD)를 연결하여 제조한 인공 전사 인자 (LacAD) 는 유핵 세포내에서 표적 유전자의 발현을 증가시켰다. LacHG에서 GAL4 DD의 linker region을 제거하였을 때 전사의 활성은 크게 증가하였고, GAL4 DD대신 leucine zipper의 DD를 사용하였을 경우에는 LacAD와 비슷한 정도로 전사 활성을 나타내었다. PR 또는 ER의 ligand binding domain (LBD)과 AD를 연결하여 만든 인공핵 수용체 (LacAPR, LacAER)는 유핵 세포에서 외부 신호에 반응하여 target 유전자의 발현을 효과적으로 조절하였다.

또한 lac operator의 대칭성 서열 [SymL(-1)]이 원래의 lac operator 서열보다 LacAD에 훨씬 잘 반응하였다.

이상의 실험을 통해 무핵 세포에서 유래한 lac repressor의 DBD를 이용하여 유핵 세포 내에서 corepressor의 존재 유무에 관계없이 결합할 수 있고 유전자의 조절을 효과적으로 조절할 수 있는 전사활성 인자와 인공핵 수용체를 만들었다.

핵심되는 말: lac repressor, DBD(DNA binding domain), GAL4 AD(activation domain), LBD(ligand binding domain)