The Journal of Microbiology, September 2002, p.205-210 Vol. 40, No. 3

P22-Based Challenge Phage Constructs to Study Protein-Protein Interactions between the σ^{54}-Dependent Promoter, *dctA*, and Its Transcriptional Regulators

Jeong Min Song[1], Eungbin Kim[2], and Joon H. Lee[1,3]*

[1]*Department of Ophthalmology and The Institute of Vision Research, Yonsei University College of Medicine, Seoul 120-749, Korea*
[2]*Department of Biology and Institute of Bioscience and Biotechnology, Yonsei University, Seoul 120-749, Korea*
[3]*Protein Network Research Center, Yonsei University, Seoul 120-749, Korea*

(Received May 14, 2002 / Accepted June 14, 2002)

To study interactions between C_4-dicarboxylic acid transport protein D and $E\sigma^{54}$ in the *dctA* promoter regulatory region, we used the challenge phage system. An *ant'-'lac* fusion was recombined onto the challenge phage, and this *ant'-'lac* fusion along with P*ant* and the *R. meliloti dctA* promoter regulatory region were cloned onto a plasmid. The plasmid bearing the *ant'-'lac* fusion was used as a reporter plasmid in a coupled transcription-translation system. Addition of purified σ^{54} to the coupled system specifically repressed transcription of the plasmid-borne *ant'-'lac* fusion. When DCTD was added along with σ^{54} to the coupled system, transcription of the *ant'-'lac* fusion was even further repressed, suggesting that DCTD may stabilize closed complexes between $E\sigma^{54}$ and the *dctA* promoter.

Key words: challenge phage system, σ^{54}, *dctA*, DCTD

Bacteria generally have one major housekeeping factor and several alternative factors, which together mediate differential gene regulation in response to various environmental signals (Gross *et al.*, 1992). For instance, σ^{54} (σ^{N}) is an alternative factor required for the expression of several genes whose products are involved in diverse metabolic functions (Gussin *et al.*, 1986; Artz and Broach, 1975). In contrast to σ^{70}-RNA polymerase holoenzyme ($E\sigma^{70}$), which is the major form of RNA polymerase in *E. coli*, σ^{54}-RNA polymerase holoenzyme ($E\sigma^{54}$) always requires an activator protein to initiate transcription. To activate transcription, the activator binds to sites about 100 base pairs upstream of the transcriptional start site and makes contact with $E\sigma^{54}$ bound at the promoter via a DNA loop. The activator catalyzes the isomerization of closed complexes between $E\sigma^{54}$ and the promoter to open complexes that are transcriptionally competent (Popham *et al.*, 1989). The isomerization requires ATP hydrolysis by the activator (Austin and Dixen, 1992; Weiss *et al.*, 1991).

C_4-dicarboxylic acid transport protein D (DCTD) activates transcription from *dctA* and belongs to a family of σ^{54}-dependent transcriptional activators, which encodes a C_4-dicarboxylic acid transport protein in *Rhizobium*. DCTD, like other σ^{54}-dependent activators, presumably contacts $E\sigma^{54}$ in order to activate transcription. However, the nature of these protein-protein contacts is not fully understood although chemical cross-linking studies with purified proteins have shown that DCTD interacts with ^{54} and the β-subunit of RNA polymerase (Lee *et al.*, 1995). We previously constructed a challenge phage to analyze interactions between *dctA* promoter and DCTD (or $E\sigma^{54}$) (Kim *et al.*, 2000).

P22, a temperate phage of *Salmonella typhimurium*, involves two regions, *immC* and *immI* that regulate the decision between lysis and lysogeny (Susskind and Botstein, 1978). The *immC* region encodes the c2 repressor, which establishes and maintains lysogeny by regulating transcription from P_L and P_R. The *immI* region contains three genes; *ant* (antirepressor), *arc* (antirepressor control), and *mnt* (maintenance of lysogeny) and modulates the activity of the c2 repressor. The Ant protein binds to the c2 repressor and prevents it from repressing transcription from P_L and P_R (Susskind and Botstein, 1978). Both the Arc and Mnt proteins repress transcription of *ant*. The *arc* gene is transcribed along with the *ant* gene from the P_{ant} promoter in the early stage of infection. Arc binds to an operator site (O_{arc}), which overlaps P_{ant}, preventing further transcription. The Mnt protein binds to a second operator that overlaps P_{ant} (O_{mnt}) to further prevent the expression of the *ant* gene (Susskind and Botstein, 1978).

* To whom correspondence should be addressed.
(Tel) 82-2-361-8461; (Fax) 82-2-312-0541
(E-mail) joon613@yumc.yonsei.ac.kr

Here, we describe application of this phage to the investigation of protein-protein interactions between DCTD and $E\sigma^{54}$.

Materials and Methods

Plasmids

The plasmids used in this study are listed in Table 1. To construct pJHLC4, pBAD22 was digested with *Eco*RI and *Hin*dIII. For cloning *S. typhimurium ntrA* gene into pBAD22, the following oligonucleotides were used: 5-CTGAGAATTCAATATGAAGCAAGGTTTGCAAC-3 and 5-CAAATTCACGCAAAGCTTCAGTAA-3. These primers create unique *Eco*RI and *Hin*dIII sites at the 5'end and in *orf95* downstream of *ntrA* gene from pJES82, respectively. These primers were used to amplify the *dctA* promoter regulatory region by polymerase chain reaction (PCR). After PCR amplification, the amplified DNA fragments (1.5 kb) were digested with *Eco*RI and *Hin*dIII and cloned into the same sites of pBAD22 to create plasmid pJHLC4.

Construction of ant'-'lac phage

Challenge phage containing *ant'-'lac* fusions was constructed by homologous recombination between phage and the *ant'-'lac* bearing plasmid pMS580 (Fig. 1). Plasmid pMS580 contains the *ant'-'lac* fusion gene and a deletion of gene *9* which encodes P22 tail protein. An overnight culture of *S. typhimurium* strain MS1883 bearing plasmid pMS580 was infected with either the challenge phage bearing the *dctA* promoter regulatory region in which the promoter overlapped P_{ant} or the challenge phage bearing the *dctA* promoter regulatory region in which the UAS overlapped P_{ant}. Cells and phage were incubated at room temperature for 20 min, then incubated at 37°C for 4 h after adding 2.5 ml of LBEDO-broth. LBEDO-broth was made by adding 2 ml 50×E-salts (10 g magnesium sulfate heptahydrate, 10 g citric acid monohydrate, 500 g dipotassium hydrogen phosphate, and 175 g sodium dihydrogen phosphate dihydrate in one liter of deionized water) and 1 ml glucose to 100 ml LB. After adding chloroform, cultures were centrifuged and the resulting supernatant liquids were transferred to sterile screw-cap tubes. An overnight culture (100 µl) of *S. typhimurium* strain MS1883 was incubated with 20 µl of 1:1,000,000 dilution of phage lysate (10^7 pfu), in the presence of purified P22 tail protein. After a 20 min incubation at room temperature, cells and phages were mixed

Table 1. Plasmids used in this study

Plasmid	Relative phenotype/genotype	Source
pSA04	*S. typhimurium ntrA* in p*lacI*^{q}POAmp^{r}Tet^r	this study
pMS580	pBR322 P_{lac} *arc-amH1605ant-lac*ZYA *Δ9a1*	M. Susskind
pMC1403	pBR322 with *E. coli lacZYA* lacking promoter and SD sequence, Amp^r	M. Casadaban
pBAD22	pBAD18 bearing *ara*C, P_{BAD} promoter, and optimized SD sequence and ATG start codon	L. Guzman
PJHLC3	pACYC184 with 1.7 kb *Eco*RI/*Sal*I fragment bearing *dctD*	J.H. Lee
pJHLC4	pBAD with 1.5 kbp *Eco*RI/*Hin*dIII fragment bearing *ntr*A	this study
pJHLC5	pMC1403 with 3 kbp *Sac*I/*Sma*I fragment bearing p22*dct*Ap	this study
pJHLC6	pMC1403 with 3 kbp *Sac*I/*Sma*I fragment bearing *dct*ApUAS	this study

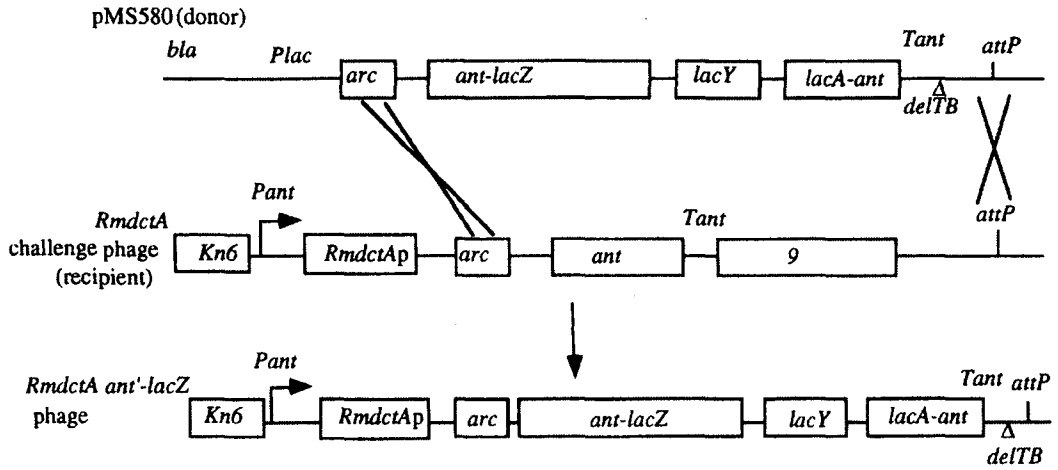

Fig. 1. Construction of P22 phage bearing the *ant'-'lac* fusion. Homologous recombination between plasmid pMS580 and P22 challenge phage generated the P22 phage bearing the *ant'-'lac* fusion. The recombination event produced tail-dependent phage, as gene *9*, which encodes the tail protein, was deleted in the recombination event. The resulting phage had the *R. melitloti dct*A promoter regulatory region in place of O_{mnt} and the *ant'-'lac* fusion, which allowed $E\sigma^{54}$ to repress transcription of the *ant'-'lac* fusion.

g agar in one liter of deionized water) and poured onto LB agar containing X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside). Recombinant phage with the *ant'-'lac* fusion produced large blue plaques, which were purified by streaking on a lawn of MS1883 in the presence of P22 tail protein. Phage lysates were prepared by growing the phage on *S. typhimurium* strain MS4032 [*leuA414*(Am) Fels-*hsdSB*(r^-m^+)] in the presence of P22 tail protein as follows. Overnight cultures were subcultured in 30 ml LB medium and grown at 37°C to OD_{650} of 0.5. A single blue plaque and 50 μl P22 phage tail were added to the culture, and after 40 h incubation, several drops of chloroform were added, and the resulting culture liquid was centrifuged at 8,000×g for 5 min to pellet the cell debris. P22 tail (100 μl) was added (5×10^{12} equivalent) to the supernatant and incubated at 37°C for 1 h to allow the tails to assemble onto tail-defective phages *in vitro*. The phages were pelleted at 17,000×g for 60 min, the resulting supernatant liquid was immediately removed, and the pellets were resuspended in 4 ml of PBS by vortexing. Phage titers were determined in the presence of P22 tail protein.

Construction of ant'-'lac lysogen

Lysogens were made with the phage bearing the *ant'-'lac* fusion in *S. typhimurium* strains MS1389 and JHL102 (same as MS1389 with *ntrA*::Tn10). Cells were grown overnight in LB medium, then infected with 10 μl (multiplicity of infection ~1) of *ant'-'lac* phage lysate along with 10 μl of P22 tail protein. After incubation at 37°C for 20 min, 0.9 ml LB medium containing 10 mM EGTA was added and the mixture incubated for an additional 20 min. The samples were then centrifuged at 17,000×g for 2 min, and pellets were resuspended in 100 μl NCE medium (197 g potassium phosphate monobasic, 250 g dipotassium hydrogen phosphate, and 175 g sodium dihydrogen phosphate dihydrate in one liter of deionized water). The resuspended cells (20 μl) were inoculated in 2.5 ml of NCE medium containing 50 μg/ml kanamycin. Cells were incubated at 37°C for 40 h, and 50 μl culture was plated on lactose-MacConkey agar containing kanamycin. Plates were incubated at 37°C overnight and lysogens were selected by the bright red color of the colonies. To construct *ntrA*$^-$ strains, *ntrA209*::Tn10 was introduced into all the *ant'-'lac* lysogenic strains by P22-mediated transduction from *S. typhimurium* strain SK284 (*hisF645 ntrA209*::Tn10). Transductants were selected on LB medium supplemented with L-glutamine (5 mM) and tetracycline. The Gln$^-$ phenotype of transductants was confirmed by streaking them on LBEDO medium agar supplemented with acid-hydrolyzed casamino acid (ACH) and LBEDO medium agar supplemented with ACH and 5 mM L-glutamine.

Construction of ant'-'lac reporter plasmid

Challenge phage containing *ant'-'lac* fusions were purified as described (Maloy and Youderian, 1994). To construct plasmids containing either *dctA*p(dctA promoter region) *ant'-'lac* fusion (pJHLC5) or *dctA*UAS (reverse direction of *dctA*p) *ant'-'lac* fusion (pJHLC6), pMC1403 bearing *lac* gene was digested with *Sac*I and *Sma*I. About 3 kbp *Sac*I-*Sma*I fragment bearing either P22*dctA*p *ant'-'lacZ* or P22*dctA*UAS *ant'-'lacZ* obtained from purified *ant'-'lacZ* phage DNA (P22*dctA*p or P22*dctA*UAS) was inserted into these sites to create pJHLC5 and pJHLC6, respectively.

In vitro transcription-translation assay

Coupled transcription-translation assays were performed to moniter the β-galactosidase activity as an indication of gene expression from *ant'-'lac* fusion as described (Hoover *et al.*, 1990). S30 extracts were prepared from *S. typhimurium* strain SK419 [*ΔntrAΔ*(*glnA ntrB ntrC*) *relA*$^-$ *hisT*$^-$] (Santero *et al.*, 1989). Plasmids pJHLC5 and pJHLC6 were purified with a Qiagen plasmid purification kit (Qiagen Inc., Germany). Coupled transcription-translation assays were performed in a total volume of 50 μl as described previously (Santero *et al.*, 1989), with the exception that no ppGpp was used. Plasmid template (pJHLC5 or pJHLC6) was added to a final concentration of 15 nM. Purified σ^{54}(Lee *et al.*, 1994) was added at various concentrations ranging from 0 to 3 μM. Purified DCTD proteins (Lee *et al.*, 1994), including wild-type DCTD, $DCTD_{L143}$, and $DCTD_{L143}$ mutant G220V were added as indicated in the text. G220V is a mutant form of $DCTD_{L143}$ that is unable to activate transcription from *dctA* and has a substitution of glycine-220 to valine. Reaction mixtures were incubated at 30°C for 3 h, after β-galactosidase activity was determined at 30°C. The level of β-galactosidase synthesized in the coupled reaction was determined for the entire 50 μl of the reaction mixtures.

Results and Discussion

Use of ant'-'lac fusions to examine interactions between DCTD and Eσ^{54} at the dctA promoter regulatory region in vivo

If DCTD stabilized closed complexes between Eσ^{54} and the *dctA* promoter, then cells that overexpressed both DCTD and σ^{54} would show higher frequencies of lysogeny than cells that only overexpressed σ^{54} when infected with challenge phage P22 *dctA*p. Plasmid pJHLC4 is a pBAD22 derivative and was constructed such that *ntrA* was under control of the *araBAD* promoter, which allowed the expression of *dctD* to be induced with arabinose. When cells harboring pJHLC4 were infected with challenge phage P22*dctA*p, no lysogens were obtained at any of the arabinose concentrations that we tested. These data suggested that the level of overexpression of σ^{54} from pJHLC4 was relatively low.

To investigate protein-protein interactions between DCTD and σ^{54}, *S. typhimurium* containing both pJHLC3 (Kim *et*

al., 2000) and pJHLC4 were infected with challenge phage P22*dctA*p. When both proteins were induced in these cells by the addition of arabinose (0.2%) and IPTG (50 nM), there was no increased lysogeny regardless of the direction of the phage construct (data not shown). When DCTD and σ^{54} were overexpressed in *S. typhimurium* from pJHLC3 and pSA4 respectively (expression of both *dctD* and *ntrA* inducible with IPTG on these plasmids), the frequency of lysogeny upon infection with challenge phage P22*dctA*p was lower than what was observed for cells in which only σ^{54} was expressed. These data suggested that the presence of both plasmids resulted in lower expression of *ntrA* from pSA4.

An *ant'-'lac* fusion was recombined onto the challenge phage P22dctAp as described in Fig. 1. This phage was then used to construct lysogens in either *ntrA*$^+$ or *ntrA209*::Tn10 strain that was otherwise isogenic. Lysogens with the *ant'-'lac* fusions were Lac$^+$ in both genetic backgrounds. The levels of β-galactosidase activity from the *ntrA*$^+$ strain were approximately two-fold lower than that from the *ntrA*$^-$ strain (Fig. 2).

We then determined that the presence of DCTD affected the expression of the *ant'-'lac* fusion in these lysogens. We reasoned that if DCTD stabilized the closed complex between Eσ^{54} and the *dctA* promoter (Lee *et al.*, 1994), we should observe decreased expression of the *ant'-'lac* fusion in the presence of DCTD. For these experiments we used the wild-type DCTD proteins and a mutant form of $DCTD_{L143}$ that was unable to activate transcription. $DCTD_{L143}$ is a constitutively active form of DCTD that

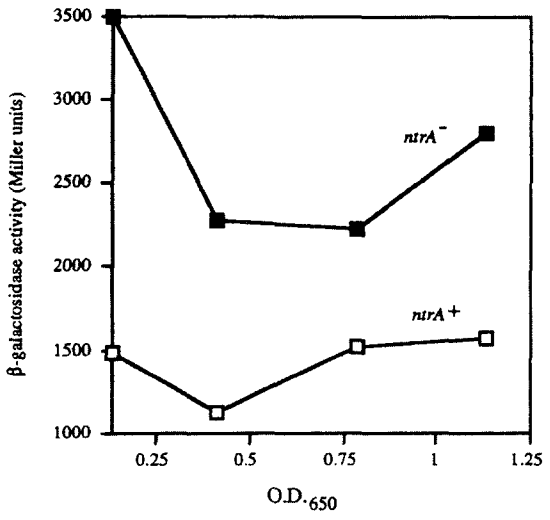

Fig. 2. β-Galactosidase activities for *S. typhimurium* lysogens with *ant'-'lac* fusions. JHL101 (open square; *ntr*A$^+$ strain) and JHL102 (closed square; *ntr*A209::Tn10 strain) were assayed for β-galactosidase activities at various cell densities. Overnight cultures were subcultured in LB medium (1:100) and grown at 37°C with vigorous shaking. Samples were withdrawn at the indicated optical densities (OD_{650}). β-galactosidase activities were determined as described by Miller (Miller, 1972). Values represent an average of two independent assays for each point.

Table 2. Effect of different DCTD proteins on expression of *ant'-'lac* fusions in *S. typhimurium* strains that either have σ^{54} or are deficient for σ^{54}

	β-galactosidase activity (nmoles ONP formed per min)	
	$+\sigma^{54}$	$-\sigma^{54}$
DCTD proteins		
None	1168	2075
Wild-type DCTD	1408	2779
$DCTD_{L143G220V}$	1050	1777

Overnight cultures of each strain were subcultured in LB medium (1:100) and samples withdrawn at several optical densities (OD_{650}; 0.15, 0.4, 0.8). β-galactosidase activities were determined as described by Miller (Miller, 1972). Values represent an average of two independent assays.

lacks the N-terminal domain and has intrinsic ATPase activity. The $DCTD_{L143}$ mutant had a substitution of glycine-220 for valine (protein referred to as G220V). This mutant was selected for its ability to channel a challenge phage that carries a single DCTD-binding site in place of O_{mnt} toward lysogeny, suggesting that the protein binds to its site normally. We selected wild-type DCTD and G220V for these experiments because they can be crosslinked to σ^{54} and the β-subunit of RNA polymerase. These proteins, however, cannot activate transcription, and so we did not have to be concerned about open complexes being formed at the *dctA* promoter in these experiments. The overexpression of wild-type DCTD and G220V in the lysogens with *ant'-'lac* fusions did not result in decreased expression from the *ant'-'lac* fusion (Table 2). These data suggested that DCTD might not stabilize the binding of Eσ^{54} to the *dctA* promoter. Alternatively, the concentrations of DCTD and Eσ^{54} inside the cell might not have been optimal for observing interactions between these proteins *in vivo*.

Use of ant'-'lac fusions to examine interactions between DCTD and Eσ^{54} at the dctA promoter regulatory region in vitro

An advantage of the *in vitro* assay was that it allowed us to alter the levels of DCTD and σ^{54} at will. Plasmid pJHLC5 carries the *ant'-'lac* fusion and the *dctA* promoter regulatory region with the *dctA* promoter overlapping P_{ant}, while pJHLC6 carries the *ant'-'lac* fusion and the *dctA* promoter regulatory region with the UAS overlapping P_{ant}. These plasmids were used in a coupled transcription-translation system in the presence of various concentrations of σ^{54} and DCTD proteins. β-Galactosidase activities were then measured to assess the level of expression of the *ant'-'lac* fusion in the coupled assay. These assays allowed us to determine the level of repression of transcription of the *ant'-'lac* fusion by DCTD and Eσ^{54} in the coupled system.

When purified σ^{54} was added to the coupled system

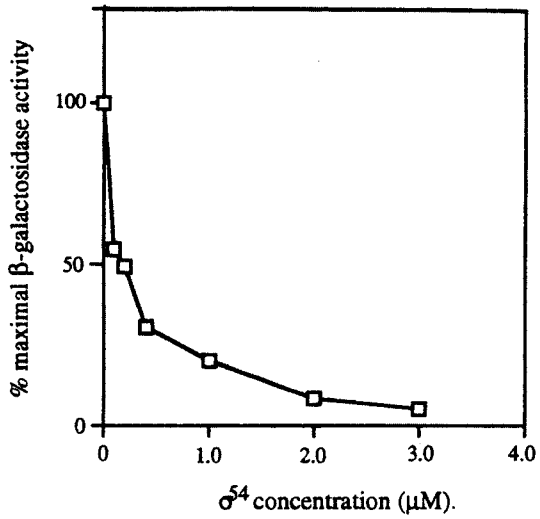

Fig. 3. Effect of σ^{54} on the expression of the *ant'-'lac* fusion carried on plasmid pJHLC5 in the coupled system. Purified σ^{54} was added to the coupled system (0, 0.1, 0.4, 0.8, 1.0, 2.0, 3.0 μM) that had pJHLC5 as the reporter plasmid. Plasmid pJHLC5 carries the *ant-lac* fusion and the *R. meliloti dct*A promoter regulatory region in place of O_{mnt}. The *dct*A promoter overlaps P_{ant}, and so $E\sigma^{54}$ can repress transcription of the *ant'-'lac* fusion. The β-galactosidase activity corresponding to 100% activity was 12 nmoles ONPG hydrolyzed/min for the entire reactive volume (50 μl) of the coupled assay. Addition of σ^{54} had no effect on expression from a *lacUV5* reporter gene indicating that the inhibitory effect of σ^{54} on expression of the *ant'-'lac* fusion did not result from σ^{54} competing with σ^{70} for binding to core RNA polymerase.

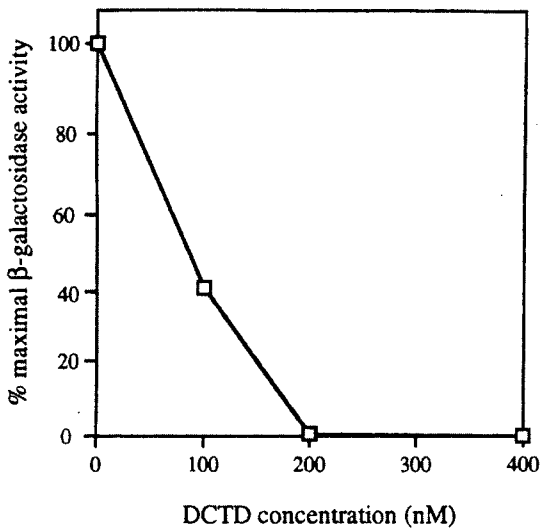

Fig. 4. Effect of wild-type DCTD on the expression of the *ant-lac* fusion carried on plasmid pJHLC6 in the coupled system. Purified DCTD was added to the coupled system that had pJHLC6 as the reporter plasmid at the following final concentrations (0, 0.1, 0.2, or 0.4 μM). Plasmid pJHLC6 carries the *ant'-'lac* fusion and the *R. meliloti dct*A promoter regulatory region in place of O_{mnt}. The UAS of the *dct*A promoter regulatory region overlaps P_{ant}, and so DCTD can repress transcription of the *ant'-'lac* fusion. The ß-galactosidase activity corresponding to 100% activity was 8.5 nmoles ONPG hydrolyzed/min for the entire reactive volume (50 μl) of the coupled assay. Addition of DCTD had no effect on expression from a *lacUV5* reporter gene, indicating that the inhibitory effect of DCTD on expression of the *ant'-'lac* fusion was specific.

with pJHLC5 as the reporter plasmid, β-galactosidase activities decreased with increasing σ^{54} concentrations (Fig. 3). In contrast, addition of σ^{54} to the coupled system using pJHLC6 as the reporter plasmid had no effect on the expression of the *ant'-'lac* fusion. When purified $DCTD_{L143}$ was added to the coupled system with pJHLC6 as the reporter plasmid, β-galactosidase activities decreased by $DCTD_{L143}$ in a dose-dependent manner, but addition of $DCTD_{L143}$ to the coupled system in which pJHLC5 was the reporter plasmid, had no effect on the expression of the *ant'-'lac* fusion (Fig. 4).

We used this *in vitro* repression assay to determine if DCTD stabilized closed complexes between $E\sigma^{54}$ and the *dctA* promoter. Using pJHLC5 as the reporter plasmid in the coupled system, σ^{54} was added to a final concentration of 100 nM, a concentration that partially (~40%) repressed transcription of the *ant'-'lac* fusion (Fig. 3). DCTD proteins were included in these coupled assays containing σ^{54} to determine if expression of the *ant'-'lac* fusion was further repressed. For these assays, either wild-type DCTD or G220V was added to the coupled assays. These DCTD proteins are unable to activate transcription, and so open complex formation was not a factor that needed to be considered in these experiments. When DCTD proteins were added to this coupled system along with σ^{54}, expression from the *ant'-'lac* fusion on plasmid pJHLC5 was slightly

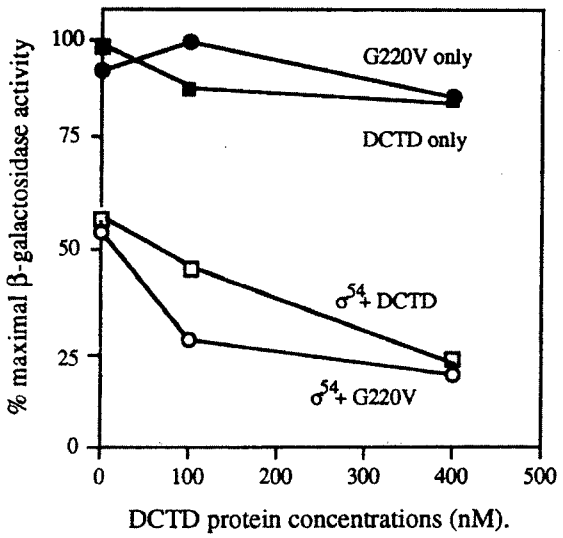

Fig. 5. Effect of DCTD and σ^{54} on the expression of the *ant'-'lac* fusion on pJHLC5 in the coupled system. Purified wild-type DCTD or G220V (circles) was added to the coupled system in either the presence (open symbols) or absence of σ^{54} (100 nM final concentrations, closed symbols). The β-galactosidase activities with wild-type DCTD and G220V corresponding to 100% activity were 11.4 and 10.6 nmoles ONPG hydrolyzed/min for the entire reactive volume (50 μl) of the coupled assay, respectively.

more repressed than when just σ^{54} was added to the assay (Fig. 5). Adding just the DCTD proteins to the coupled system did not affect expression from the *ant'-'lac* fusion on pJHLC5. Taken together, these data suggested that the DCTD proteins may have stabilized the closed complex between $E\sigma^{54}$ and the *dctA* promoter in these *in vitro* repression assays, although the effects of the DCTD proteins on enhancing the $E\sigma^{54}$-mediated repression of the *ant'-'lac* fusion were relatively small.

In conclusion, the *in vivo* assays did not give any indication that DCTD stabilizes binding of $E\sigma^{54}$ to the *dctA* promoter. We could control the concentrations of both DCTD and σ^{54} more easily in the coupled system, however, and we did get an indication that DCTD might stabilize the closed complex at *dctA* promoter. Although the degree to which DCTD enhanced repression of the *ant'-'lac* fusion by $E\sigma^{54}$ in the coupled system was not big enough to be exploited to examine further interactions between DCTD and $E\sigma^{54}$, these assays might still be useful for examining interactions between other σ^{54}-dependent activators and $E\sigma^{54}$. A good candidate is NTRC, which stabilizes closed complexes between $E\sigma^{54}$ and the *K. pneumoniae nifL* promoter (Minchin *et al.*, 1989). E^{54} apparently has a lower affinity for the *nifL* promoter than it doesfor the *dctA* promoter, as closed complexes between $E\sigma^{54}$ and the *dctA* promoter were readily detected in footprinting experiments (Lee *et al.*, 1994), but closed complexes between $E\sigma^{54}$ and the *nifL* promoter were detected only in the presence of NTRC (Minchin *et al.*, 1989). In addition, the binding sites for NTRC are farther from the *nifL* promoter than from the *dctA* promoter (by ~30 bp). This longer distance between the activator binding sites and the promoter will probably provide greater flexibility for the intervening DNA, and may allow NTRC to contact $E\sigma^{54}$ more readily than DCTD.

Acknowledgments

This study was supported in part by a faculty research grant of Yonsei University for 2001 and a research grant for SRC (Protein Network Research Center, Yonsei University) from the Korea Science and Engineering Foundation (KOSEF).

References

Austin, S. and R. Dixon. 1992. The prokaryotic enhancer binding protein NTRC has an ATPase activity which is phosphorylated and DNA dependent. *EMBO J.* 11, 2219-2228.

Gross, C.A., M. Lonetto, and R. Losick. 1992. Transcription regulation, p. 129-176. *In* S.L. McKnight and K.R. Yamamoto (eds), Bacterial sigma factors. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Hoover, T. R., E. Santero, S. Porter, and S. Kustu. 1990. The integration host factor stimulates interaction of RNA polymerase with NIFA, the transcriptional activator for nitrogen fixation operons. *Cell* 63, 11-22.

Kim, E., D. Kim., and J.H. Lee. 2000. P22-based challenge phage constructs to study DNA-protein interactions between the σ^{54}-dependent promoter, *dctA*, and its transcriptional regulators. *J. Microbiol.* 38, 176-179.

Lee, J.H. and T.R. Hoover. 1995. Protein cross-linking studies suggest that *Rhizobium meliloti* DCTD, a σ^{54}-dependent transcriptional activator interacts with σ^{54} and the β-subunit of RNA polymerase. *Proc. Natl. Acad. Sci.* USA. 92, 9702-9706.

Lee, J.H., D. Scholl, B.T. Nixon, and T.R. Hoover. 1994. Constitutive ATP hydrolysis and transcription activation by a stable, truncated form of *Rhizobium meliloti* DCTD, a σ^{54}-dependent transcriptional activator. *J. Biol. Chem.* 269, 20401-20409.

Maloy, S. and P. Youserian. 1994. Methods in Molecular genetics, p 205-233. Academic Press, Inc., San Diego, California.

Popham, D. L., D. Szeto, J. Keener, and S. Kustu. 1989. Function of a bacterial activator protein that binds to transcriptional enhancers. *Science* 243, 629-635.

Santero, E., T.R. Hoover, J. Keener, and S. Kustu. 1989. In vitro activity of the nitrogen fixation regulatory protein NIFA. *Proc. Natl. Acad. Sci.* USA. 86, 7346-7350.

Susskind, M.M. and D. Botstein. 1978. Molecular genetics of bacteriophage P22. *Microbiol. Rev.* 42, 385-413.

Weiss, D. S., J. Batut, K. E. Klose, J. Keener, and S. Kustu. 1991. The phosphorylated form of the enhancer-binding protein NTRC has an ATPase activity that is essential for activation of transcription. *Cell* 67, 155-167.