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Application of *Escherichia coli* UP Element in Gram-positive Industrial Bacteria

Baek Rak Lee

School of Biotechnology and Biomedical Science, Inje University, Gimhae, 621-749, Korea

A+T rich DNA sequences referred to as UP elements, a component of some bacterial promoters, locates upstream of the -35 hexamer and increases rate of transcription initiation dramatically by interacting with RNA polymerase α-subunit. *Escherichia coli* RNA polymerase(RNAP) α-subunit contains two independently folded domains. The N-terminal domain is responsible for dimerization and interactions with β and β' and the carboxy-terminal domain(α-CTD, 245-329) contains the DNA binding determinants. Genetic analyses have identified amino acid residues in α-CTD critical for DNA binding and UP element function. These residues are essential for cell viability in *E. coli* and are nearly invariant in bacteria and therefore the DNA sequences recognized by are also likely to be highly conserved. The extent of stimulation by UP element varied widely from less than two fold to 90-fold in naturally occurring UP elements in *E. coli*. Furthermore UP elements are not limited to only *E. coli* promoters. The extreme evolutionary conservation of the amino acids in α-subunit of RNAP involved in UP element interaction suggests that UP elements are prevalent in promoters throughout the bacterial world.

However it is not known whether various RNAP α binds to DNA or the presence and functional recognition of *E. coli* UP element in all bacteria. Therefore we investigated the recognition and function of the *E. coli* UP elements *in vitro* as well as *in vivo* conditions in various industrial bacteria such as Bacilli, Corynebacteria and Streptomyces. These informations obtained have broad implications on gene expression and regulation and possibility of sharing promoter sequences in heterologous bacterial kingdom.

First, We tested in *C. ammoniagenes*, a well known nucleoside producer strain, whether *C. ammoniagenes* α-subunit of RNAP(Cα) could bind *E. coli* UP element DNA fragments with electrophoretic mobility shift assays. *C. ammoniagenes* α did not shift *E. coli* proximal, or full consensus UP element oligonucleotides, while *E. coli* α shifted them efficiently under the condition we used. To evaluate in vivo functions, we tried complementation experiments using the *E. coli* HN317 strain which is a *rpoA*^{ts} mutant at the nonpermissive temperature. *C. ammoniagenes* α did not restored viability to the *rpoA*^{ts} mutant at the nonpermissive temperature. It's complementation ability was similar to the *E. coli* R265A mutant which is defective in UP element activation. Therefore purified Cα could not interact with *E. coli* UP element *in vitro* and could not replace function of Eα *in vivo*. However, reconstituted RNAP holoenzyme from Cα and *E. coli* β, β', and sigma 70 subunits interacted efficiently with *E. coli* UP element. This fact suggests that α-CTD of *C. ammoniagenes* is functioning properly when assembled with the other parts of RNAP from *E. coli*.

Second, we tested in *C. glutamicum* which is a well known amino acid producer and evolutionally neighbor bacterium *with C. ammoniagenes*. Its alpha contained 338 amino acid residues and revealed higher homology to *C. ammoniagenes*(82%), *Mycobacterium tuberculosis*(70%) and *E. coli* (42%) (Table 1). Comparative sequence and pattern analysis revealed that it had highly conserved domains typical for alpha subunit of eubacteria. *C. glutamicum* alpha contained F262, R265, N268, C269, G296 K298, S299 residues which has been known important for DNA binding and UP element function. *C. glutamicum* did shifted *E. coli* proximal, or full consensus UP element oligonucleotides and efficiently restored viability to the rpoA^{ts} mutant at the nonpermissive temperature. These results suggest *C. glutamicum* RNAP preserves ability of UP element recognition.

Third, high G+C, Gram-positive bacteria of the genus *Streptomyces* undergo a complex morphological and physiological differentiation and various antibiotics are synthesized concomitantly with the development. These differentiation processes involve a wide range of regulatory mechanisms involving alternative sigma factors, transcription factors. We demonstrate here UP element activation by RNAP in *Streptomyces*. *E. coli rrnB* P1 UP element increased multiple round transcription by similar level with three different RNA polymerase; *E. coli* RNA polymerase, *S. coelicolar* RNA polymerase as well as reconstituted hybrid RNA polymerase. *Sce* α did not bind detectably to the UP element DNA fragments at concentrations up to 4 M while *E.coli* α binds specifically to this DNA fragment with an apparent equilibrium constant of $1x10^{-7}$ M. *Sce* α able to restore viability to the *rpoA*^{ts} mutant at the nonpermissive temperature. From these results we conclude that *Sce* RNA polymerase able to recognizes the UP element and increase transcription as a holo enzyme, even though *Sce* α failed UP element DNA binding in condition we used. We also measured extent of gene expression by a naturally occurring *E. coli rrn*B P1 UP element or by a consensus *E. coli* UP element using a transcriptional fusion to promoterless *xylE* gene of *Pseudomonas putida*. We found the consensus UP element increased activity of *rrn*D P2 promoter of *S. coelicolor* dramatically in *S. coelicolor* as well as in *S. venezuelae*.

Table. 1 Comparison of homology of RNAP-α operon in representative bacteria.

Strains	S13	S11	S4	RNAP α	L17
C. ammoniagenes	122	134	201	334(100%)	Not analyzed
C. glutamicum	122(89%)	134 (85%)	201 (86%)	338 (82%)	163
E. coli	118 (56%)	129 (57%)	206 (46%)	329 (42%)	127
S. coelicolor	126 (64%)	134 (78%)	204 (41%)	340 (69%)	168
M. tuberculosis	124 (78%)	139 (76%)	201 (74%)	347 (70%)	180
B. subtilis	121 (57%)	131 (67%)	200 (48%)	314 (49%)	120
P. polymyxa	Not analyzed	131 (69%)	Not exist	314 (50%)	121

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