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Mass Spectrometric Screening of Transcriptional Regulators: Identification of Transcriptional Regulator Involved in Antibiotic Biosynthesis in *Streptomyces coelicolor*

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The identification of transcriptional regulators has been a key issue in molecular biology and numerous investigations have been reported about it. Recently developed DNA pull-down strategies are rapid and versatile in identifying proteins that bind to a specific DNA sequence (1-2). In this report, we propose an improved DNA affinity capture assay (DACA) combined with mass spectrometry for one-step identification of DNA-binding transcriptional regulators affecting an operon in bacteria. The introduction of liquid chromatography-nanospray tandem mass spectrometry (nLC-MS/MS) has enabled direct analysis of large protein complexes or even total cellular proteins with a single preparation (3). This technology also allows us to identify overall proteins captured on the beads conjugated with DNA promoter sequences. As the captured proteins on the bead include usual abundant nonspecific binding proteins as well as specific binding proteins, we can get some insights to modify and improve the DACA.

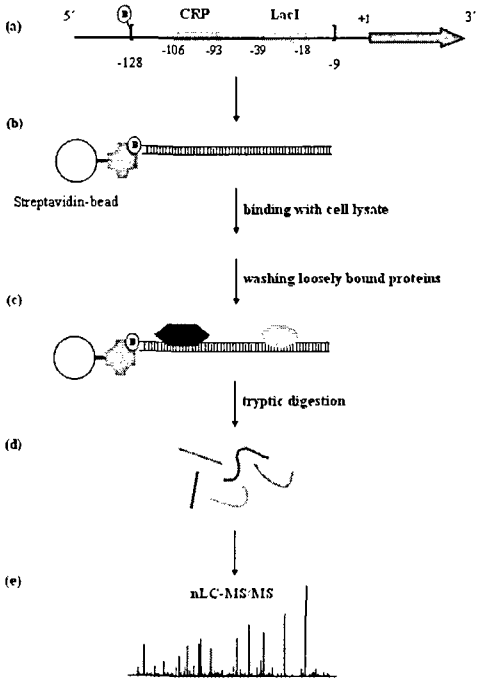

Fig. 1 Schematic view of the procedure of DNA-affinity capture assay.

The *lac* promoter (P_{lac}) of *Escherichia coli* was used as a model system. We used a cell lysis buffer containing 500 mM NaCl to dissociate all DNA-binding proteins from chromosomal DNA, and pre-incubated cell lysate with competitor DNA before introducing target DNA to reduce nonspecific bindings (Table 1). Using these procedures, *lac* operon repressor (LacI) and cyclic AMP receptor protein (CRP) were successfully identified as well-known DNA-binding regulators.

Streptomycetes are Gram-positive soil bacteria which produce a lot of secondary metabolite and undergo unique morphological and physiological differentiation. *Streptomyces coelicolor* A3(2) is the genetically best-characterized streptomycete and the genome sequence of it was completed. This strain produces at least four

Table. 1 Detection level of LacI and CRP at various conditions

Condition	Score[a] : %C[b]		Concentration (mg/mL) of		NaCl[c]	Pre-incubation[d]
	LacI	CRP	ssDNA	poly(dI-dC)		
A	-	14.1 : 8.6	0.1	0.1	×	×
I	-	40.3 : 17.6	0.1	0.1	O	×
B	-	20.3 : 18.1	0.5	0.5	O	×
C	-	30.5 : 25.7	0.02	0.02	O	×
II	30.2 : 14.7	120.3 : 56.7	0.1	0.1	O	O

[a] SEQUEST score
[b] Sequence coverage of the protein
[c] 500 mM NaCl was added to the cell lysis buffer.
[d] Cell lysate was pre-incubated with competitor DNAs for 15 min at ice.

distinctive antibiotics, two of which are pigmented; red tripyrolle undecylprodigiosin (Red) and blue polyketide actinorhodin (Act). Biosynthetic gene clusters for these two antibiotics have been isolated and well characterized (4,5). These gene clusters have their own pathway-specific transcriptional activator, *redD* and *actII-ORF4*, whose transcriptions increase dramatically during the transition from exponential to stationary phase in liquid culture, followed by transcription of the corresponding biosynthetic genes and production of Red and Act (6,7). It has been shown that antibiotic production in streptomycetes had close correlations to other cellular processes, suggesting that the antibiotic production might be regulated at several levels (Fig. 2).

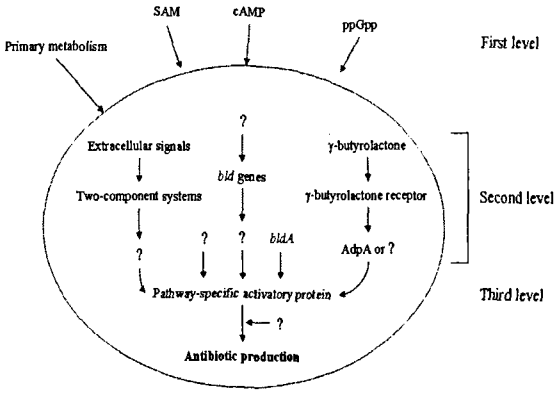

Fig. 2 Overview of the regulations of antibiotic production.

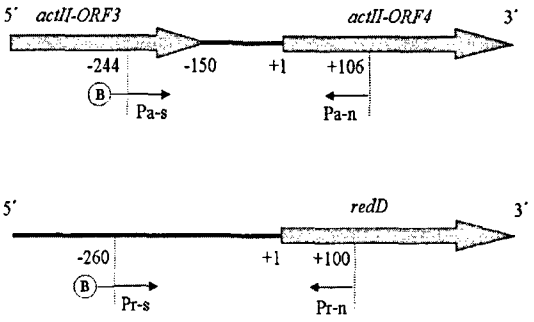

Fig. 3 Promoter regions of *actII-ORF4* and *redD* used in this study.

The developed DACA was applied to identify unknown transcriptional regulators involved in actinorhodin (Act) and undecylprodigiosin (Red) biosynthesis in *Streptomyces coelicolor*, which was focused on the transcriptional regulators bound to the promoter region (Fig. 3) of pathway-specific transcriptional activator, *actII-ORF4* and *redD*. Putative transcriptional regulators were identified through mass spectrometric analyses of bound proteins (Table 2 & 3). The binding of five regulators to the promoter region of *actII-ORF4* or *redD* were reexamined using electrophoretic mobility shift assay (EMSA). Some regulators were further investigated using gene overexpression and deletion studies. Overexpression of the genes triggered diverse effects on the levels and kinetics of antibiotics productions. AdpA and SCO6008 delayed and inhibited Red

biosynthesis but increased Act production. SCO5405 stimulated earlier Red and Act biosyntheses and increased Act production. SCO3606 showed an inhibition of only Red biosynthesis. On the other hands, SCO1480, SCO5552, and SCO3932 showed no significant effect on antibiotic production. Specially, a null mutant of SCO6008 failed to produce Act on medium lacking proline. From above results, it is proposed that AdpA, SCO5405, and SCO6008 are closely involved in the regulation of antibiotics biosyntheses.

Table. 2 Identified proteins binding to *actII-ORF4* promoter region

Gene No. (cosmid-based gene No.)	Score : %C	Description
SCO2792 (SCC105.23)	60.2 : 10.1	AdpA, AraC-family transcriptional regulator
SCO0310 (SC5G9.19c)	40.1 : 12.5	Putative TetR-family transcriptional regulator
SCO3932 (SCQ11.15c)	30.2 : 12.4	Putative GntR-family transcriptional regulator
SCO5405 (SC8F4.09c)	28.2 : 27.2	Putative MarR-family transcriptional regulator

Table 3. Identified proteins binding to *redD* promoter region

Gene No. (cosmid-based gene No.)	Score : %C	Description
SCO2792 (SCC105.23)	170.3: 31.0	AdpA, AraC-family transcriptional regulator
SCO5405 (SC8F4.09c)	40.2 : 39.9	Putative MarR-family transcriptional regulator
SCO5552 (SC1C2.33c)	170.1 : 28.2	Putative IclR-family transcriptional regulator
SCO6008 (SC7B7.05)	90.3: 44.4	Putative transcriptional repressor
SCO3606 (SC66T3.17)	30.2: 15.8	Putative regulator
SCO3859 (SCH69.29)	240.4: 62.6	Putative DNA-binding protein
SCO1480 (SC9C5.04c)	30.2: 31.8	Conserved hypothetical protein

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