

Development of an ON/OFF-transcriptional control system for plural genes to express sequentially

Yuri Samata

s13mb107@mail.saitama-u.ac.jp

Kei Asai

asai@mail.saitama-u.ac.jp

ADivision of Life Science, Graduate School of Science and Engineering, Saitama University,
255 Shimo-Ohkubo, Sakura-ku, Saitama, Saitama 338-8570, Japan.

Keywords: Sigma factor, transcription, stress response

The gene expression in either a bacterial cell or a eucaryotic cell is important bio-technology performed frequently. Generally expression is induced by adding an inducer, which is usually hard to be inactivated or removed, and depression after induction is difficult.

Sigma factors rule the DNA-binding specificity of RNA polymerase to the promoter, and are a conserved protein family among bacteria. The protein family of sigma factors is further divided into a subfamily, Extra-Cytoplasmic Function (ECF) sigma factors. The activity of ECF sigma factors is regulated by binding specifically to cognate anti-sigma factor proteins on a simple principle by protein-protein interaction. Anti-sigma factor proteins localize to the cytoplasmic membrane and response to various external and environmental stress.

ON/OFF control of gene expression is thought to be performed reversibly by some environmental stress (temperature, pH, the oxidation state, etc.). We have tried to use these plural sigma-anti-sigma control systems in order to control a lot of gene clusters consecutively.

In *Bacillus subtilis*, a Gram-positive sporulating soil bacterium, seven ECF sigma factors have been identified. The SigW protein is a well-studied ECF sigma factor in *Bacillus subtilis*. It is regulated by anti-sigma factor, RsiW. Their coding genes constitute an operon, whose transcription is induced in response to various cell-wall antibiotics, alkaline shock, and other stresses affecting cell-envelope homeostasis. SigM, is strongly involved in cell envelope integrity and regulated with YhdL and YhdK anti-sigma proteins. Expression and activity of SigM are elevated under acid, heat, salt, superoxide and cell envelope stresses.

We investigated whether the SigW and SigM could be used for ON/OFF control system by using pH exchange of medium used for *B. subtilis* cell culturing.