Gene expression profile analysis for circadian promoter activities of cyanobacterial bioluminescent reporter strains using non-metric multidimensional scaling

Tokitaka Oyama¹, Hiroshi Ito², Takao Kondo³, Y-h. Taguchi⁴

Keywords: Cyanobacteria, Circadian rhythm, bioluminescent reporter, promoter, non-metric multidimensional scaling method

1 Introduction.
Circadian rhythms in photosynthetic organisms are important features in their living forms, since the usage of solar energy in the day phase is an essential process for them. Approximately, during half of day, no solar energy is available, thus it is useful to alter cellular activities through regulation of gene expression during this period. The circadian rhythm of cyanobacteria is maintained by three clock genes (kaiA, kaiB, kaiC), and the circadian oscillation can be reconstructed by mixing the three purified proteins with ATP in a test tube[2]. However, it is uncertain how the expression of individual genes is coordinated under the control of the KaiABC clock[1]. In this poster, we would like to clear out how the genetic network is regulated with and without circadian rhythms by using expression profiles of a bioluminescent reporter system. This system enables us to measure gene expression activities of each promoter of living cells with a high time resolution. The obtained time sequential gene expression profiles are analyzed by using non-metric multidimensional scaling method (nMDS)[3, 4].

2 Method
We have generated bioluminescent reporter strains carrying luciferase reporter genes under putative promoter regions of each ORF of Synechococcus elongatus PCC 7942. Bioluminescence of living cells is measured automatically under continuous light conditions. In this time, we have measured 764 ORFs sequentially aligned in the genome (c.a. 30 % of total genome) and found c.a. 700 clones exhibit reliable bioluminescence. Obtained gene expression profiles are analyzed by nMDS and the relationships between genes are shown as two dimensional alignment.

3 Results
After analyzing gene expression profiles by nMDS, we have found that circular gene alignment is obtained when we make use of partial gene expression profiles. In Fig. 1 (a), we have shown that two dimensional nMDS embedding of time interval from 31 hours to 41 hours, from 55

¹Dev. Biol. Sci., Nagoya Univ., and CREST, JST, Japan. E-mail: oyama@bios11.bio.nagoya-u.ac.jp
²Dev. Biol. Sci., Nagoya Univ., and CREST, JST, Japan. E-mail: hito@bio.nagoya-u.ac.jp
³Dev. Biol. Sci., Nagoya Univ., and CREST, JST, Japan. E-mail: kondo@bio.nagoya-u.ac.jp
⁴Dept. Phys., Chuo Univ., E-mail: tag@granular.com
to 65, from 81 to 90, and from 105 to 115, by using sign-reversed correlation coefficients between gene expression profiles as dissimilarity. Circular arrangement of genes is obvious. In order to understand the meanings of circular arrangement, we have shown in Fig. 1(b) the first and second principal components obtained by principal component analysis (PCA) for gene standardized expression profiles. Clearly, these are parts of sinusoidal forms. This suggests that most of promoters exhibit sinusoidal oscillation although during some period these activities are inhibited.

In order to confirm the above conjecture, we have applied the following procedure to gene expression profiles. (i) Standardization of (zero mean, variance of unity) gene expression profiles. (ii) De-trending up to quadratic polynomial function of time. (iii) Computation of the mean profile. (iv) Removal of mean from all gene expression profiles. (v) Applying normalized PCA[4] to gene expression profiles. The resulting first and second principal components turn out to be class 1(peak at dust)/class 2(peak at dawn) and that orthogonal to these, respectively. Since mean profiles describe the activity changes between day/night time, We concluded that gene expression profiles consist of both the basic day/night activity changes and individual sinusoidal activities (e.g., class 1 and class 2).

References


