【Grant-in-Aid for Specially Promoted Research】
Science and Engineering (Chemistry)

Title of Project : Creation of genome manipulation technology using super restriction enzyme

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Research Area : Chemistry related to living body

Keyword : Nucleic acid, Genome analysis, Biotechnology, Recombination

【Purpose and Background of the Research】

It is now well recognized that the genomes of higher animals and plants are never simple aggregates of many genes. Rather, these genes and their regulation factors are placed at predetermined sites in the genomes and communicate each other for precise regulation. Apparently, a method to manipulate genomes in a predetermined fashion is essential to understand biological functions of genomes and further employ them for our practical needs. However, there have been few tools to manipulate huge genomes, and accordingly there has been little information on the detailed functions of genome systems.

We already developed man-made tool (Artificial Restriction DNA Cutter; ARCUT) which cuts huge double-stranded DNA at desired site. The objectives of this project are to complete ARCUT-based method of genome manipulation, and also to clarify the functions of genome systems. The "genome manipulation technology", which is constructed here, could alter the basic concepts of conventional biotechnology.

【Research Methods】

We will first establish the method to manipulate huge genomes using ARCUT. Then, this technology is used for precise analysis of the function of gene(s) in genomes. Specifically, we cut genomes at the target site, and clarify the communication of genes (and also with the regulation factors) in genomes. The following four approaches are attempted. (1) Alteration of a target gene to a desired gene through homologous recombination which is promoted by ARCUT-induced site-selective scission of genome, (2) silencing of a target gene also through homologous recombination, (3) segregation of a gene from other genes by cutting a predetermined site of genome by ARCUT, where the influence of this genome manipulation on biochemical properties (transcription efficiency, protein localization in cell, etc.) is quantitatively assessed, and (4) clipping of a portion of genomes by ARCUT, which is analyzed in detail in vitro by spectroscopic and other physicochemical means.

【Expected Research Achievements and Scientific Significance】

In the present project, the method of genome manipulation is provided for the first time. Undoubtedly, this method should dramatically accelerate the progresses of new biotechnology (e.g., drug discovery). In the current biotechnology, external genes are mostly introduced to cells by using vectors, where these genes should primarily work independently from each other. Thus, the mutual communications of genes and regulation cofactors cannot be appropriately evaluated. In extreme cases, the best gene for the therapy of a disease, for example, could be thrown away in the screening process, simply because of the lack of appropriate mutual communications with other genes and regulation cofactors in the cells. The present work provides straightforward solutions to these problems. Of course, the genome manipulation technique should be directly applicable to gene therapy which is one of the most challenging topics.

【Publications Relevant to the Project】

[1] "Artificial restriction DNA cutter for site-selective scission of double-stranded DNA with tunable scission-site and specificity", M. Komiyama, Y. Aiba, Y. Yamamoto, J. Sumaoka, *Nature Protoc., 3*, 655-662 (2008).

[2] "Homologous Recombination in Human Cells using Artificial Restriction DNA Cutter", H. Katada, H. J. Chen, N. Shigi, M. Komiyama, *Chem. Commun.*, 6545-6547 (2009).

【Term of Project】 FY2010-2014

【Budget Allocation】 400,400 Thousand Yen

【 Homepage Address and Other Contact Information】

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