【Grant-in-Aid for Scientific Research(S)】
Science and Engineering (Chemistry)

Title of Project： Control and elucidation of gene expression at a molecular level

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Research Area：Chemical Biology
Keyword：Gene Regulation, DNA nanostructure, SAHA polyamide conjugate

【Purpose and Background of the Research】

In life sciences, understanding the control of gene expression by histone modification and cytosine demethylation is currently of intense interest. However, detailed mechanisms of such epigenetic modifications of biomolecules have not been well understood. In this study, the following aims will be pursued to understand better the control of epigenetic gene expression at a molecular level.

1) To achieve control of cell initialization and differentiation through epigenetic gene activation by sequence-specific DNA-binding molecules.

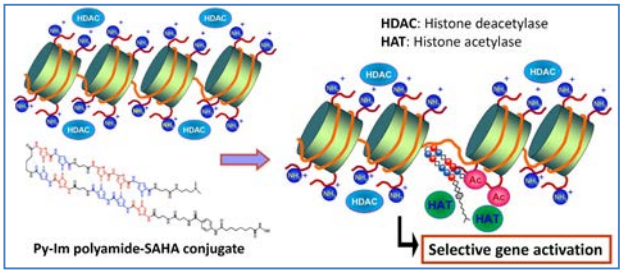
2) To develop new methods of controlling gene expression by designing molecules that recognize DNA structure.

3) To develop a technique for direct imaging and analysis of enzymes and reactions related to gene regulation.

【Research Methods】

Development of Py-Im polyamides that induce cell reprogramming.

It is known that iPS cells are pluripotent and therefore have promise in the development of key technologies for regenerative medicine. Recent advances in cell biology demonstrate that



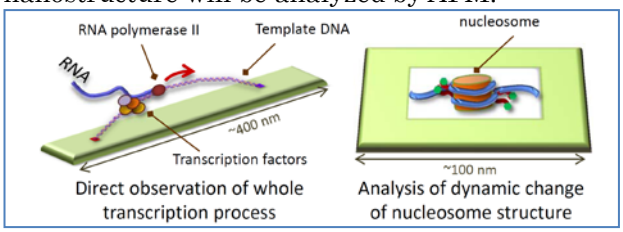
epigenetic modification, such as DNA methylation and histone deacetylation, can control gene expression. Indeed, an inhibitor of histone deacetylase (HDAC), suberoylanilide hydroxamic acid (SAHA), is known to effectively cause histone acetylation to activate the gene expression.

We have prepared a library of SAHA-Py-Im polyamide conjugates that are known to bind DNA in sequence-specific fashion. We have selected SAHA polyamides that effectively upregulated important genes for induction of iPS cells, such as Nanog and Oct-3/4, in mouse embryonic fibroblasts (MEF). We also confirmed an increase in the level of histone acetylation at the promoter region of these genes.

In this project, we will extend the screening library in an attempt to find SAHA-Py-Im polyamide conjugates that activate a set of genes involved in the reprograming of human cells such as Oct-4, Sox-2, and Klf4. We will further investigate histone acetylation and DNA methylation.

Direct visualization of enzymes and proteins related to gene expression

For the direct imaging of the eukaryotic transcription processes, the complex formation and dynamic behavior of general transcription factors and RNA polymerase will be observed by high-speed atomic force microscopy (AFM) in the designed DNA nanostructure. To clarify the enzyme behaviors associated with epigenetic gene expression, nucleosomes placed in the DNA nanostructure will be analyzed by AFM.



【Expected Research Achievements and Scientific Significance】

The combination of our research approaches will allow us to control and visualize the gene expression and clarify the mechanism of epigenetic gene expression.

【Publications Relevant to the Project】

・G. N. Pandian, H. Sugiyama *et al.*, *ChemBioChem,* 2012, **13**, 47 – 50
・A. Rajendran, M. Endo, H. Sugiyama, *Angew. Chem. Int. Ed.* 2012, **51**, 874 – 890

【Term of Project】FY2012-2016
【Budget Allocation】163,700 Thousand Yen
【Homepage Address】
http://kuchem.kyoto-u.ac.jp/chembio/