【Grant-in-Aid for Scientific Research (S)】

**Broad Section G**

# Title of Project： Regulation of Enhanceosome by Cohesin

SHIRAHIGE Katsuhiko
(The University of Tokyo, Institute for Quantitative Biosciences, Professor)

Research Project Number: 20H05686 Researcher Number：90273854
Keyword：Chromosome hyperstructure, transcriptional elongation, ATP motors, cohesins, enhanceosomes

## 【Purpose and Background of the Research】

Cohesin is a protein complex that plays a central role in the regulation of chromosomal high order structure in eukaryotic cells, and has recently been shown to have a motor activity that introduces a loop structure into DNA in an ATP-dependent manner. Although cohesins function as sister chromatid adhesins, we have shown that cohesins bind to insulator sequences and enhancer regions of genes, and that one of the roles of cohesins is to regulate the transcriptional elongation of genes. Their roles are closely related to known transcriptional elongation regulators such as bromodomain protein BRD4 and super elongation complex AFF4, suggesting that they play important roles in developmental and differentiation control and cancer malignant transformation. In recent years, it has been reported that an amorphous, dynamic, large protein network controlled by "liquid-liquid phase separation" is formed on some enhancer DNAs, and it is also required to reconsider the complex (enhanceosome) on the enhancer as a collection of weak interactions. The objective of the present application is to understand the regulation of transcription elongation by enhanceosomes in molecular terms, with a particular focus on cohesin, and to clarify its physiological significance.

## 【Research Methods】

The following four approaches are taken. (1) Elucidation of the cohesin function in the enhancer using in vitro reconstitution: We have already succeeded in reconstitution of the enhancer using HeLa nuclear extract. Quantitative Western blot and mass spectrometry are used to comprehensively analyze the amount of integration factors and modification of cohesin and NIPBL. This point is also evaluated, as there may be differences in DNA conformation by cohesin removal or mutation. The resulting enhanceosome is isolated and directly visualized with an atomic force microscope (high-speed AFM). Investigate whether the structure of the enhanceosome is dynamically altered or whether cohesin induces the change. (2) Functional analysis of cohesin through high-resolution visualization of the nuclear enhanceosome: To complement the results obtained in vitro in (1), analysis using a genomics technique targeting the intracellular enhancer is performed. (3) Comprehensive identification of proteinaceous and nonproteinaceous components of the enhanceosome: The enhanceosome is a large complex of diverse proteins. Because there is a high possibility that there are still unidentified enhanceosome components, a new method is used in combination with mass spectrometry to comprehensively identify the proteins present in the enhanceosome. (4) Cohesin modification and participation in transcription reaction: In the reaction system of (1), phosphorylation modification and acetylation modification of cohesin and NIPBL are examined, and the participation in the enhanceosome control is examined.

## 【Expected Research Achievements and Scientific Significance】

Mutations in cohesin/NIPBL, AFF4, and BRD4 are known to cause developmental abnormalities. It is also involved in carcinogenesis, mainly in the blood cell system. The regulation of transcription elongation by enhanceosomes is expected to play an essential role in the selection of differentiation pathways and the maintenance of differentiation status. The results of this study are expected to greatly contribute to understanding these diseases at the molecular level. Anticancer agents targeting epigenetic pathways have recently been developed and used. Transcriptional elongation control pathways may also be targets for anticancer drugs. The basic understanding of the enhanceosome provided by this research will be a foundation for future drug discovery.

## 【Publications Relevant to the Project】

· Izumi K, Nakato R, ... Shirahige K*, Krantz ID* (*shared corresponding authors). Germline gain-of-function mutations in AFF4 cause a developmental syndrome functionally linking the super elongation complex and cohesin. **Nat Genet.** 47:338-344, (2015).
· Deardorff MA, Bando M, Nakato R,...Shirahige K.* HDAC8 mutations in Cornelia de Lange syndrome affect the cohesin acetylation cycle. **Nature.** 489:313-317, (2012)

【Term of Project】FY2020- 2024

【Budget Allocation】151,800 Thousand Yen

【Homepage Address and Other Contact Information】
http://www.iam.u-tokyo.ac.jp/chromosomeinformatics/