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

# Title of Project： Molecular architecture of vertebrate centromeres

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Research Area： Chromosome Dynamics
Keyword： Chromosome segregation, Chromosome function, Epigenetics

## 【Purpose and Background of the Research】

Chromosome segregation during mitosis is critical to transfer genetic information to daughter cells in all organisms. If errors of chromosome segregation occurred, chromosome instability would be caused. Therefore, it is the most important topic in genetics to understand mechanisms for faithful chromosome segregation.

The centromere is a key dynamic interface with microtubules from the mitotic spindle. To establish a functional centromere, a subset of centromere proteins must assemble on DNA. Defining the molecular mechanisms by which centromere proteins specify the position on the chromosome and drive centromere assembly remain key goals. In this project we aim to define the molecular mechanisms for centromere specification and assembly.

## 【Research Methods】

To perform this research project we need to take various approaches including a chromosome engineering method, biochemical analysis, and structural biology by X-ray crystallization analysis. We will perform following three projects in parallel.

I) Centromere specification and assembly

A centromere is usually specified at a particular locus on each chromosome. However, as a rare case, a new centromere is formed on non-centromere locus upon inactivation of original centromere (neocentromere formation). To understand mechanisms how (neo)centromeres are specified we will try to establish an experimental system by which neocentromeres were generated in chicken DT40 cells. In addition, we will try to create artificial centromeres by combination of the experimental neocentromere with the LacI-LacO system (Figure 1).

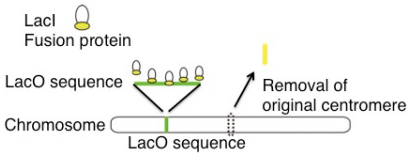

Figure 1 Generation of artificial centromere

II) Reconstitution of centromere protein complexes

The centromere is composed of DNA and various proteins to form a large complex. To understand architecture and function of the large centromere complex we must characterize feature of each protein. We will express and reconstitute centromere protein (sub)-complexes. Then, we evaluate function of these sub-complexes based on DNA or microtubule binding activities.

III) Structural analysis of centromere protein complexes

The reconstituted protein complexes will be applied to X-ray crystallization analysis. Once we will determine crystal structure of the protein complexes, we will make mutant DT40 cells to disrupt complex formation and evaluate biological significance of the complex formation.

## 【Expected Research Achievements and Scientific Significance】

Combined our current knowledge for centromere structure and function with results in this project we expect to clarify vertebrate centromere architecture in detail. As we can perform various experiments in a single Lab, we are standing a good position to lead the research-field of centromere biology. In future it may be possible to develop drug design based on the structures of centromere protein complex.

## 【Publications Relevant to the Project】

・Nishino T et al., CENP-T-W-S-X forms a unique centromeric chromatin structure with a histone-like fold. Cell, 148, 487-501 (2012).
・Hori T et al., CCAN makes multiple contacts with centromeric DNA to provide distinct pathways to the outer kinetochore. Cell, 135, 1039-1052 (2008).

【Term of Project】FY2013-2017

【Budget Allocation】166, 000 Thousand Yen

## 【Homepage Address and Other Contact Information】

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