別紙様式第４

学　位　論　文　要　旨

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［論文題名］

RbAp48 is essential for viability of vertebrate cells and plays a role in chromosome stability

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［要　旨］

In eukaryotic nuclei, the genomic DNA is wrapped around histone octamers to form nucleosome core particles, basic repeating units of chromatin, with the aid of histone chaperone proteins through replication-coupled or replication-independent pathways. Since nucleosomal histones are thought to serve as a barrier to prevent transacting factors involved in DNA metabolism from accessing DNA, histone chaperone proteins play important roles in chromatin assembly, DNA replication, transcriptional regulation and DNA damage repair. Chromatin assembly factor-1 (CAF-1), a well-conserved protein complex consisting of three polypeptides of p150, p60, and p48, was originally purified from human cells as a factor that promotes *de novo* nucleosome assembly on replicating SV40 DNA. Of these, p48 subunit, initially identified as a retinoblastoma (Rb)-binding protein (RbAp48), is a member of the histones H3/H4 chaperons containing WD40 repeats β-propeller structure. In addition to roles in nucleosome assembly process, both RbAp48 and RbAp46 are also found in several other complexes involved in the regulation of chromatin structure, including NuRD (nucleosome remodeling histone deacetylase complex), histone deacetylase (HDAC)-containing complexes (Sin3) and histone methyltransferase (HMT)-containing complex. Since RbAp48/p46 proteins have the ability to bind directly to histones H4 and H3, they are proposed to act as escorting factors that connect these complexes to their substrate nucleosomes, and are implicated in multiple aspects of cellular events involved in histone metabolizing process depending on the complex' s composition. Physiological functions of RbAp46/48 family have been well studied in model organisms such as yeast, plants, *Drosophila*, and mammals. Still, their *in vivo* functional relevance is yet unclear.

In order to examine the biological role of pRbAp48 in chicken DT40 cells, we generated a tetracycline-inducible system for conditional RbAp48-knokout cells. In the absence of tet, the growth rate of the conditional *RbAp48*-knockout cells was similar to that of *RbAp48* $^{-/+}$, *tetHAp48* clone and DT40 cells. Upon the addition of tet, *RbAp48*-knockout cells began to grow slowly at 36 h, and finally, almost all of the cells were dead by 72-84 h, indicating

that RbAp48 is essential for viability of DT40 chicken B cells. Loss of RbAp48 caused the delay in S phase progression accompanied by impaired DNA replication, followed by accumulation in G2/M phase, and finally leading to cell death. Prior to cell death, these cells exhibited aberrant mitosis such as highly condensed and abnormal chromosome alignment on the metaphase plate, leading to chromosome missegregation. Depletion of RbAp48 also caused dissociation of heterochromatin protein 1 (HP1) from pericentromeric heterochromatin. Furthermore, depletion of RbAp48 from cells led to elevated levels of acetylation and slightly decreased levels of methylation, specifically at Lys-9 residue of histone H3.

In conclusion, we speculate that the crucial roles of RbAp48 in cell viability and proliferation in S phase is most likely due to its indispensable role in CAF-1 activity, and RbAp48-guided chromatin regulatory complexes other than CAF-1 may also confer pericentromeric domains with a functional configuration to recruit heterochromatin binding proteins such as HP1 and other partner molecules via histone modification, which is critical for chromosome integrity. Our findings may help to understand the potential link between replication-coupled nucleosome assembly, DNA replication, chromosome segregation and histone modification in vertebrate cells.

備考　論文要旨は、和文にあっては 2，000 字程度、英文にあっては 1，200 語程度とする。