

## 学位論文抄録

### Generation of immunoglobulin V-region somatic hypermutation by GANP in germinal center B-cells

(胚中心機能分子GANPによる免疫グロブリンV領域遺伝子体細胞突然変異誘導機構)

シン シャイレンドラ クマール  
Singh Shailendra Kumar

熊本大学大学院医学教育部博士課程医学専攻発生・再生医学  
研究者育成コース

指導教員

阪口 薫雄 教授  
熊本大学大学院医学教育部博士課程医学専攻免疫学

## Abstract of the Thesis

**Background and Purpose:** Chromatin modification occurring throughout the *immunoglobulin (Ig) gene-locus* is required for active transcription, and somatic hypermutation (SHM) of the *IgV-region* loci to achieve the secondary B-cell diversification. During immune responses, B-cells diversify their *Ig-gene* by the activation-induced cytidine deaminase (AID). The purpose of this study is to elucidate the molecular mechanism for AID targeting towards the transcriptionally active open chromatin at the *IgV-region* locus during SHM.

**Methods:** To address this issue, we analyzed the specific associated proteins with GANP in the nucleus by proteomics analysis. Chromatin immunoprecipitation analysis was carried out to characterize nucleosome occupancy and specific pattern of histone modifications, transcription-coupled events, and SHM at *IgV-gene* locus by GANP expression.

**Results:** GANP forms a complex with AID and translocates into the nucleus. The histone acetyltransferase (HAT) region of GANP controls the AID targeting to the *IgV-region* DNA. GANP is also involved in the enhancement of active chromatin markers of H3K9ac and H3K4me3 and transcription elongation to the downstream *IgV-region* gene locus with RNA Pol-II at serine 2 phosphorylation. GANP collaborates with histone H1 and enhances acetylation at lysine 63 (H1K63ac), suggesting the regulatory effect of GANP on the linker function of histone H1. GANP co-precipitates with RNA Pol-II and its stalling factor Spt5 that also interacts with AID, indicating their recruitment to *IgV-region* is under the transcription-coupled manner. Interestingly, GANP is selectively recruited at the middle of the rearranged *IgV-region* segment with the interference of nucleosome occupancy, mediated by HAT-domain. GANP promotes the specific chromatin modifications during the transcription-coupled events at *IgV-region*, and controls the accessibility of AID positioning at *IgV-region* gene and suggests a specific role for generation of GANP-mediated complex in antigen specific high affinity B-cells.

**Conclusions:** This study identified the GANP-interacting proteins in B-cell nuclei by a proteomics strategy and uncovered the function of GANP through the analysis of its associated protein in the regulation of chromatin organization and active transcription at the *IgV-region*.