Kyoto University	Doctoral degree (Genomic Medicine),	Name	Bo Hu
Dissertation Title	3D epigenome dynamics in normal and stalled development (正常および遅延発生における 3D エピゲノムダイナミクス)		

（Dissertation Summary）

Counted among the most complex machineries known to man, the cells that make up all living organisms lie at the foundation of life itself. Beyond traditional means of grossly assessing cellular morphology and composition, recent advances in sequencing-based assays have fuelled tremendous progress in understanding biological processes across varying scales. In particular, the importance of regulatory mechanisms that does not involve variation in actual genetic sequences – “epigenetics” – has become increasingly evident given their critical function in fine-tuning DNA compaction and folding. The dynamic epigenomic landscape thus not only underlies the diversity between cell types with specialized functions, but also distinguishes healthy and pathological states. Through the assembly of a 3D epigenome atlas of mouse germline development, we found that repressive domains and enhanced insulation maintains transcriptional integrity in the face of global DNA de-methylation and pervasion of enhancer-like signatures during epigenetic reprogramming in primordial germ cells. Subsequently in spermatogonia, these insulatory restraints and then removed en masse as global euchromatization and peripheral detachment of chromatin takes place in the preparation for meiotic entry. On the other hand, we leveraged a compendium of 3D epigenomic profiles in brain tumours to reveal that a specific histone mutation, H3K27M, specifically leads to the formation of repressive loop structures via a reader of H3K27me3, cPRC1. Following the validation of H3K27M-associated cPRC1 loops’ impact in primary patient tumours, we further pinpointed this process as a therapeutic vulnerability – with the application of a cPRC1 inhibitor demonstrating the capacity to alleviate the oncogenic differentiation blockade. This thesis details how the systematic application of integrative multi-omics can dissect molecular determinants of health and disease as well as provide actionable insights towards the future development of targeted therapeutic strategies.

(Summary of Dissertation Examination Results)

The candidate has clearly presented two contrasting lines of investigation into the mechanisms through which proper three-dimensional epigenome remodeling facilitates healthy development as well as the pathogenic outcomes of defective nuclear architecture in diverse contexts such as brain tumors and neurodevelopmental disorders. Novel findings in the context of germ cell differentiation include pinpointing variable insulation as a critical regulator of gametogenesis: whereas the division of large topologically associating domains into smaller units safeguards transcriptional fidelity in primordial germ cells (PGCs), more differentiated germline stem cells leverage reduced barrier strength to achieve long-range activation of gametogenic programs. It was also uncovered that higher-order genome organization proceeds in a globally unidirectional manner during PGCs-to-spermatogonia development, culminating in spermatogonia possessing largely de-condensed genome with minimal attachment to the nuclear periphery, rounding out a comprehensive description. On the other hand, it was found that the dichotomy of genome patterning through diffuse versus confined histone H3 lysine 27 tri-methylation is modulated by several disease-relevant mutations, with constriction noted as a hallmark of progenitor-like cells. Subsequently, the breadth of such domains was associated with the concentration of canonical Polycomb Repressive Complex 1, which in turn governs distal repressive chromatin looping. Through implicating dysregulated polycomb looping at developmental regulators as a prevailing disease mechanism, potential therapeutic agents were identified and validated in high-grade gliomas, paving the path for further pharmaceutical development.

This thesis substantiates the candidate’s extensive and wide knowledge of bioinformatics, demonstrates expert research capability in the field of chromatin, cancer, and developmental biology and presents new discoveries and concepts that contribute to the profound understanding and further development of the candidate’s research field. Moreover, the thesis is written logically and coherently, which satisfies the degree requirement that the thesis shall serve as a valuable document for future reference.

As a result of the oral examination held on 2022/06/30 regarding the dissertation contents and related matters, we certify that the candidate has passed the oral examination.