論文 / 著書情報
Article / Book Information

題目(和文)	
Title(English)	Spatiotemporal Dynamics of H2AX Phosphorylation in Immediate Response to DNA Double-strand Breaks
著者(和文)	TrakarnphornsombatWatanya
Author(English)	Watanya Trakarnphornsombat
出典(和文)	学位:博士(理学), 学位授与機関:東京工業大学, 報告番号:甲第12489号, 授与年月日:2023年6月30日, 学位の種別:課程博士, 審査員:木村 宏,岩﨑 博史,田口 英樹,藤田 尚信,加納 ふみ
Citation(English)	Degree:Doctor (Science), Conferring organization: Tokyo Institute of Technology, Report number:甲第12489号, Conferred date:2023/6/30, Degree Type:Course doctor, Examiner:,,,,
学位種別(和文)	博士論文
Category(English)	Doctoral Thesis
種別(和文)	論文要旨
Type(English)	Summary

（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

系・コース： Department of, Graduate major in	Life Science and Technology 系 コース	申請学位（専攻分野）： Academic Degree Requested	博士 (Science) Doctor of
学生氏名： Student's Name	Watanya Trakarnphornsombat	指導教員（主）： Academic Supervisor(main)	Hiroshi Kimura
		指導教員（副）： Academic Supervisor(sub)	

要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

DNA double-strand break (DSB) is one of the dangerous events for genomic stability. A failure of DSB repair could result in genetic mutations, and eventually cell death and diseases, such as cancer. In response to DSB, histone H2AX is phosphorylated at serine 139 by members of the phosphatidylinositol 3-kinase-related kinase (PIKK) family kinases, including ataxia telangiectasia-mutated (ATM), ataxia telangiectasia and Rad3-related (ATR), and DNA-dependent protein kinase (DNA-PK). These kinases have a similar domain organization with redundant and distinct functions. Different phenotypes were observed in knockout mice of these genes. ATM-knockout mice are sterile and have T cells deficiency, while DNA-PK-knockout mice are fertile but have severe combined immunodeficiency. ATM is reported to be a major kinase for the formation of phosphorylated H2AX (γ-H2AX), but the immediate-early kinetics of the γ-H2AX formation in response to DSBs remains unknown. In this study, to reveal the mechanism of H2AX phosphorylation, Fab-based live endogenous modification labeling (FabLEM) was used for visualizing its accumulation in ATM-proficient and -deficient cells. In addition, contributions of another histone modification that might play a role in DSB repair, H4 lysine 16 acetylation (H4K16ac), and its acetyltransferase, males absent of the first (MOF), on γ-H2AX formation were investigated.

Fabs specific for the γ-H2AX were introduced into ATM-proficient (11-4) and -deficient (AT5BIVA) cells. DNA damages were induced by microirradiating a nuclear area with a 405-nm laser line and the accumulation of γ-H2AX at the damaged area was monitored using a confocal microscope by time-lapse and live-cell imaging. To study the functions of the DNA-PK in the immediate-early DSB, a DNA-PKinhibitor, NU7441, was applied. To examine the effect of MOF on DSB, MOF was knocked down by expressing shRNA with a lentivirus system. Kinetics and chromatin binding ability of ATM and DNA-PK were analyzed using a permeabilized cell assay by treating cells with a non-ionic detergent, TritonX-100, before laser irradiation, and also using fluorescence recovery after photobleaching (FRAP) with a 488-nm laser line.

After laser microirradiation, γ-H2AX-specific Fabs were accumulated in the irradiated areas within a few minutes both in ATM-proficient and -deficient cells. As the accumulation kinetics were similar, ATM did not appear to be essential for the immediate early γ-H2AX formation. In contrast, when the DNA-PK inhibitor was applied, the accumulation of γ-H2AX was slightly delayed and prolonged, suggesting that ATM is dispensable for the early DSB response and the DNA-PK is an essential kinase.

In MOF-knockdown cells, the accumulation of ATM, but not γ-H2AX, at the DNA damage lesions was affected. After permeabilization to extract diffusible proteins, γ-H2AX accumulated only in permeabilized 11-4, but not AT5BIVA cells, suggesting that, in cells without DSBs, ATM repeatedly binds to and dissociates from chromatin, whereas DNA-PK is diffusible in the nucleus free of chromatin binding. The different behaviors of ATM and DNA-PK were also observed in living cells by FRAP. ATM recovered more slowly than Ku80, a subunit of DNA-PK. After DSBs were induced, the ATM recovery became dramatically slower, suggesting that the ATM binds more tightly to the damaged chromatin. These FRAP

data are consistent with the results in permeabilized cells. MOF-knockdown had little effect on the fluorescence recovery of ATM but reduced ATM accumulation in response to DNA damages.

The relationship between three-dimensional chromatin organization and γ-H2AX formation kinetics was also analyzed because the topologically associating domain (TAD) governs DNA into loops, which might play a role in DSB repair. As TAD is regulated by the cohesin complex and the CCCTC-binding factor (CTCF), these factors were depleted from cells using the auxin-induced degron system. CTCF depletion indeed affected the early accumulation kinetics and domain expansion of γ-H2AX.

This study shows that, even though ATM is reported to be a major kinase for γ-H2AX formation in general, it is dispensable for the immediate-early DSB response as the γ-H2AX dynamics are similar between ATM-proficient and -deficient cells. DNA-PK seems to be the primary kinase in the immediate-early DSB as the inhibition of DNA-PK substantially decreases the γ-H2AX accumulation. The DNA-PK inhibition also prolonged γ-H2AX accumulation in ATM-proficient cells, which might be resulted from ATM hyperactivation in the presence of the DNA-PK inhibitor. MOF did not affect the chromatin binding of ATM but did affect ATM accumulation, suggesting that MOF might regulate the ATM activation and that both MOF and H4K16ac might function at later stages in DNA repair. In summary, for proper DSB repair response, both the early response by DNA-PK and the amplifying signals by ATM for later response are needed to maintain the genome maintenance mechanism.