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# 論文内容の要旨

Nucleosome is a fundamental unit of chromatin, consisting of 146bp of DNA wrapped around an octomer of four kinds of histones, H2A, H2B, H3 and H4. Because nucleosomes can be significant obstacles to transcription that are mediated by RNA polymerase II (Pol II), it is needed to destabilize nucleosomal structure by displacement of histones during Pol II passage. The destabilization of nucleosomes during transcription results in histone loss over the regions of heavily transcribed genes (Lee, C.K. et al. 2004). Histone loss sometimes causes aberrant transcription initiation from cryptic sites within coding regions (Kaplan, C.D. et al. 2003). To maintain the fidelity of transcription initiation, histone deposition behind Pol II passage is necessary.

Recent studies have revealed an interesting phenomenon regarding histone deposition during transcription in eukaryotes other than yeast. The phenomenon is that histone H3 variant, H3.3 is selectively incorporated into nucleosomes during transcription (Ahmad, K. and Henikoff, S. 2002a). In contrast to canonical histone H3, H3.3 is synthesized throughout the cell cycle and deposited onto DNA both during and outside of S phase (Ahmad, K. and Henikoff, S. 2002b, Tagami, H. et al. 2004). The selective deposition of H3.3 is a very interesting phenomenon, however, the underlying mechanisms as well as in vivo biological meanings have been elusive.

To elucidate the mechanisms I tried to identify a factor that mediates the deposition of H3.3. In this study I focused on a transcription elongation factor Spt6 as a candidate. Studies of yeast Spt6 have revealed that Spt6 plays a critical role in maintaining normal chromatin structure during transcription elongation interacting with histone H3 (Bortvin, A. and Winstone, F. 1996, Kaplan, C.D. 2003). Although yeast has no transcription variant of histone H3, these findings in yeast studies led me an idea that Spt6 might be involved in the variant histone H3.3 deposition during transcription in higher eukaryotes.

In order to understand the Spt6 function in higher eukaryotes, I investigated the in vivo role of Spt6 using *D. melanogaster* as a model organism. First, to investigate the localization of Spt6 on chromatin, I carried out ChIP-on-chip microarray analyses. By the analyses of ChIP-on-chip microarray, strong signals of Spt6 (probe set p-value $< 0.001$) were detected on about 30 genes within the half of the entire *Drosophila* genome (Table 1). As these 30 genes are various in their functions, expression patterns and genome structures, any common features were not found among them. In most of the 30 genes, Spt6 was distributed throughout the each gene, especially in exons (Figure 3). I selected several genes such as *heat shock protein 83* (*hsp83*), *La related protein* (*larp*) and *thread* from these Spt6-localized genes for further analyses.

To investigate the function of Spt6 in vivo I generated and characterized *spt6* null mutant. By P-element excision, I generated *spt6W40*. *spt6W40* allele has a small deletion in the second exon of the *spt6* gene that causes a stop of the protein synthesis, hence is functionally null (Figure 5-A). Homozygotes of *spt6W40* show an embryonic lethal phenotype.

To examine the effects of the *spt6* mutation on transcription, I performed RT-PCR

analyses using RNA from embryos to measure the amounts of mRNA. RT-PCR analyses with primers for 5' sides and 3' sides of *hsp83* and *larp* mRNA showed that the levels of the mRNA were increased only at the 3' sides but not at the 5' sides in the *spt6* mutant compared with the wild type (Figure 6-B). These results suggest that the transcription initiates from the middle of the coding region in the *spt6* mutant. To analyze the size of the transcripts from *hsp83*, I carried out a Northern blot analysis. The RNA probes synthesized over the coding sequence of *hsp83* detected not only expected 3 kbp transcripts but also shorter transcripts only in the *spt6* mutant (Figure 7). These shorter transcripts may be the products of aberrant transcription on *hsp83* gene in the *spt6* mutant. These results suggest that *spt6* mutation causes aberrant transcription initiation from cryptic sites within the coding region.

To examine the possibility that the aberrant transcription in the *spt6* mutant is due to changes in chromatin structure, I carried out a micrococcal nuclease (MNase) assay using *spt6W40* embryos. Although there was no difference in the patterns of bulk chromatin between the wild type and the *spt6* mutant, chromatin on *hsp83* exhibited an increased sensitivity to MNase in the *spt6* mutant compared with the wild type (Figure 8). This result indicates that the *spt6* mutation causes changes in the chromatin structure on the *hsp83* gene in vivo. Because the ChIP analyses with pan-H3 antibodies showed that the level of general H3 on *hsp83* was reduced in the *spt6* mutant (Figure 9), the changes in the chromatin structure on *hsp83* in the *spt6* mutant may be due to loss of nucleosomes.

Previously, examination of several *Drosophila* genes revealed that nucleosomes containing either canonical histone H3 or variant histone H3.3 were lost during transcription, and were selectively replaced with nucleosomes containing H3.3 (Wirbelauer, C. et al. 2005). Because the chromatin structure was changed by the loss of nucleosomes in the *spt6* mutant, I consider the possibility that Spt6 can help the deposition of H3.3 during transcription. To observe the correlation of Spt6 with H3.3, I measured the levels of H3.3 on three Spt6-enriched genes (*hsp83*, *thread* and *larp*) and two non-enriched genes (*β-tubulin* and *cyp4d21*) by the ChIP analyses. The levels of H3.3 were higher on the Spt6-enriched genes rather than non-enriched genes (Figure 10). This result suggests that there is some correlation between Spt6 and H3.3 in their localization. For further examination of physical relationship between Spt6 and H3.3, I carried out a Re-ChIP assay to examine whether Spt6 localizes on nucleosomes containing H3.3 or H3. As the results, on *larp* and *hsp83*, Spt6 was efficiently immunoprecipitated with the nucleosomes containing H3.3 rather than H3 (Figure 11A). This indicates that Spt6 preferentially localizes on the nucleosomes containing H3.3 rather than H3.

In order to examine the functional relationship between Spt6 and H3.3, I used *spt6* RNAi lines gifted by R. Ueda. When *spt6* RNAi was ubiquitously induced by an *Ay-GAL4* driver, almost all the animals died before the pupal stage (Table 2-B). If Spt6 is involved in the deposition of H3.3, a decrease in the efficiency of H3.3 deposition caused by *spt6* knockdown can be suppressed by over-expression of H3.3. To test this, H3.3 was over-expressed in the *spt6* RNAi line. As controls H3 or GFP was expressed instead of H3.3. The lethal phenotype of *spt6* knockdown was significantly suppressed only by the over-expression of H3.3. This result

suggests that Spt6 is functionally related with H3.3 in vivo.

In summary I have found that Spt6 plays an important role in maintaining the chromatin structure during transcription elongation, thereby repressing production of aberrant transcripts in *D. melanogaster*. I also observed the correlation of Spt6 with variant histone H3.3. This is the first report to suggest the relationship between Spt6 and H3.3. Further investigation will clarify the function of Spt6 in the H3.3 deposition, and it may provide new insights into chromatin regulations during transcription elongation.

論文の審査結果の要旨

遺伝子発現の調節のため，クロマチンはダイナミックにその構成や構造を変化させる．クロマチン変化の一つに，ヒストン置換，すなわち通常型とヴァリアント型ヒストンの置換がある．転写活性の低いクロマチンにはH3が多く存在し，高いところにはH3.3が分布している．このようなヒストンヴァリアントの選択的分布は，活性化型クロマチンの維持やそのエピジェネティックなマークとして貢献しているのではないかと考えられている．しかし，「H3/H3.3 の選択的分布はいかにして達成されるか？」という問題はこれまで取り上げられたことがなかった．中嶋さんは，活性化型クロマチンへの H3.3 の選択的配置に，これまで「転写伸長反応制御因子」と考えられてきたSpt6が関与するのではないか，と考え，この仮説を検証した．

Spt6 タンパク質は特定の遺伝子に局在しており，特にエクソン上に多く分布していた．Spt6 の機能欠失型変異を作成したところ，Spt6 が胚発生に必須であることが分かった．Spt6 の変異胚では，本来 Spt6 が局在している遺伝子上のヒストンが減少し，クロマチン構造が壊れており，かつ遺伝子の内部から異常な転写産物ができていた．これらの結果は，Spt6 がクロマチン構造の維持を通じて転写の精度保証を行っていることを示している．

Spt6 の H3/H3.3 の選択的分布への関与を調べるために，RNAi 法で Spt6 量を減少させたときに生じる生存率低下をヒストンの過剰発現でレスキューする実験を行った．その結果，Spt6 量減少による症状は H3 ではレスキューされないが，H3.3 の過剰発現で選択的にレスキューされることが分かった．また，Spt6 は H3 を含むヌクレオソームよりも H3.3 を含むヌクレオソームに多く存在していることも明らかになった．これらの結果は，Spt6 がヌクレオソーム上で H3.3 と選択的に結合していることを示している．

中嶋さんが行ったさまざまな分子生物学的解析や遺伝学的解析の結果から，「Spt6 は H3.3 を含むヌクレオソームの維持あるいは形成に寄与している」という新しい概念が生まれた．中嶋さんの仕事は，H3/H3.3 の選択的配置の分子機構を開拓したものとして高く評価できる．中嶋さんは，Spt6 の分布が一様でなくエクソンに多いことを発見するなど，分野の新展開の土台を作った．また，中嶋さんが作成した Spt6 の変異系統は，活性化クロマチンへの H3.3 の選択的配置の意義を解析する貴重な材料となるだろう．以上の理由で，中嶋みかげさんの論文は博士号授与の要件を満たすと審査員全員一致で判断した．