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学位記番号	総研大甲第771号
学位授与の日付	平成１６年３月２４日
学位授与の要件	生命科学研究科　遺伝学専攻 学位規則第４条第１項該当
学位論文題目	DNA supercoiling factor contributes to dosage compensation in *Drosophila melanogaster*
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In eukaryotes, genomic DNA is highly compacted (packaged) into chromatin via the nucleosome structure in which 147 bp of DNA is wrapped around a histone octamer in a left-handed superhelical turn. Chromatin creates barriers for various aspects of transactions on DNA. Therefore, alteration of chromatin structure into more dynamic state is a critical process for DNA metabolism. How is the energy for the process provided? One of the explanations for it is "generation of superhelical tortion into DNA". Indeed, resent studies have shown that an ability to generate negative superhelical torsion in DNA is shared by ATP-dependent chromatin remodeling activities which participate in the alteration of chromatin structure during gene regulation.

DNA supercoilig factor (SCF) was first identified in the silkworm as a protein that generates negative supercoils in DNA in conjunction with topoisomerase II. Subsequently, a *Drosophila melanogaster* counterpart of SCF was identified, and it was revealed that the factor interacts with topoisomerase II in the nucleus and localizes to puffs on polytene chromosomes. These findings implicate a role of SCF in transcription on chromatin.

However, these negative supercoiling activities including that of SCF were identified only by assay systems *in vitro*, and physiological functions of them remain nuclear. In this study, I aimed to determine the in vivo role of SCF to clarify the biological significance of negative supercoiling activities.

To analyze the biological function of SCF, I attempted to perform RNA interference (RNAi). Although RNAi is a powerful method for silencing genes, injection of dsRNA interferes with gene expression only transiently. In order to overcome the problem, a method to express dsRNA as an extended hairpin-loop RNA has been developed recently, and found to be successful in generating RNAi in *Drosophila.* Thus, I employed this method to achieve the knock-down of SCF function throughout development. Surprisingly, I found specific and dramatic reduction in the male biability. However, this male-specific lethality could be caused by the difference of RNAi activity between males and females. To test the possibility, I compared RNAi effect in both sexes. Western blot analysis showed that the amounts of SCF protein in both sexes are similarly decreased by RNAi, indicating that the observed male-specific lethality was not due to the difference of RNAi effect in the two sexes.

In *Drosophila,* at least five genes, *male less(mle), male-specific lethal 1, 2,*and *3(msl-1, msl-2, and msl-3),* and *male absent on the first (mof),* have been discovered on the basis of the male-specific lethal phenotype of their loss-of-function alleles. In males, the products of these genes form a complex (MSL complex) that binds to the numerous sites on the X chromosome, and mediate the dosage compensation through 2-fold enhancement of the levels of transcription on the X chromosome, relative to females. The dosage compensated male X chromosome takes a more decondensed structure relative to the autosomes, which correlates with hyper-acetylation of the lysine 16 residue of histoneH4 (H4K16). Although little is known on the molecular mechanisms underlying the hypertranscription of X-linked genes, it has been thought that the altering chromatin structure is a key element of the dosage compensation. Therefore, the observed male-specific lethality in the knock-down of SCF leads me to presume that SCF might be involved in the context of dosage compensation. To investigate this possibility, I first examined whether the knock-down of SCF affects the binding of the MSL complex and subsequent acetylation of H4K16 along the X chromosome. I performed immunostaining of polytene chromosomes to analyze the distribution of

MSL complex and acetylated H4K16. This experiment revealed that MSL complex still localizes to the X chromosome in the males deficient in the SCF function, and acetylation of H4K16 is also not affected under the condition.

If SCF is truly involved in the dosage compensation, it is expected that the reduction of SCF function results in the inappropriate transcriptional regulation of X-linked genes. In this idea, I next examined the effect of the SCF RNAi on the expression levels of X-linked genes. I performed quantitative real time RT-PCR and found the male specific reduction in the X-linked genes expression level under the SCF RNAi. These results suggest that SCF plays a role in hypertranscription of X-linked genes after the association of MSL complex and subsequent acetylation of H4K16 along the male X chromosome.

Since the gene expression analysis showed correlation between the SCF function and the dosage compensation, I investigated the possible interaction between SCF and components of the dosage compensation machinery *in vivo*. Biochemical and genetic analyses were performed, and the results obtained demonstrated that SCF and the dosage compensation complex interact both physically and functionally.

To directly examine the correlation between SCF and dosage compensation *in vivo*, I analyzed distribution of SCF on polytene chromosomes prepared from each sex. In spite of the fact that the single male X chromosome contains only a half amount of DNA as compared to the paired female X or the autosomes, SCF signals on the male X chromosome were more densely distributed along the chromosome relative to the autosomes or female X chromosome. This observation supports the idea that SCF functions as a dosage compensation regulator. Moreover, I found that overexpression of SCF on the polytene chromosome caused abnormal morphology exhibiting a bloated appearance and a loss of its banding pattern on the male X chromosome, but not the autosomes or female X chromosome. These findings suggest that SCF plays a role in the alteration of chromatin structure via the function of the dosage compensation complex.

The above analyses show that SCF participates in the dosage compensation only after the association of MSL complex and the subsequent acetylation of H4K16 by MOF histone acetyl transferase. To confirm the idea, I analyzed distribution of SCF protein on the polytene chromosomes in *mof* mutant background. Compared to the wild type background, SCF labeling was significantly reduced on the male X chromosome in *mof* mutant. By contrast, the SCF labeling on the autosomes was not affected. Thus, I conclude that MOF activity is necessary for the proper landing of SCF on the male X chromosome.

Taken together, the results reported here show that DNA supercoiling factor SCF is responsible for the hypertranscription of X-linked genes after the association of MSL complex and subsequent acetylation of H4K16 by MOF activity along the male X chromosome, and hence the SCF function is essential for the *Drosophila* dosage compensation. The present findings support the previously proposed model in which SCF plays a role in the transcriptional activation via the alteration of chromatin structure.

論文の審査結果の要旨

古橋さんの博士論文は、ショウジョウバエのスパーコイル化因子（SCF）の生体内での機能を調べたものである。SCF は DNA の負の超らせんを導入するタンパク質であり、DNA の転写や複製に重要な役割を果たしていると考えられるが、その生物学的な意義は明らかではない。古橋さんは近年ショウジョウバエで利用され始めた誘導型 RNAi の手法を適用して個体レベルで SCF の機能を調べた。

SCF 遺伝子断片の逆向き反復配列を UAS プロモーター下流につないだ vector を導入したハエと、GAL4 タンパク質を産生するハエを交配すると、その子孫個体でヘアピン型の二本鎖 RNA が転写され、SCF に対する RNAi が誘導される。発生初期より全細胞で RNAi を誘導すると、SCF タンパク質が著しく減少した。この *SCF* RNAi 変異体では雌個体は成虫までほぼ正常に発生するが、雄個体は蛹期に死亡し、雄特異的致死という遺伝子量補正異常と類似する表現型が明らかとなった。実際に X 染色体に存在する 3 種の遺伝子の発現量を調べたところ、RNAi 変異体雄では顕著な発現の低下が観察された。

ショウジョウバエでは性（X）染色体の遺伝子量補正は雄 X 染色体の転写量を倍化することによっておこなわれ、この過程では雄の X 染色体にのみ結合する MSL タンパク質複合体が histone H4 の hyper-acetylation などに重要な役割を果たしていることが知られている。そこで *SCF* 遺伝子と *MSL* 遺伝子群の遺伝学的相互作用を調べたところ、*MSL-1* 遺伝子と二重ヘテロ接合体において顕著な雄特異的致死性が検出された。実際に免疫沈降実験をおこなうと、SCF タンパク質、あるいはそれと結合している Topoisomerase II タンパク質に対する抗体により、MSL 複合体の 5 種のタンパク質がともに沈降してきた。すなわち、*SCF* 遺伝子と *MSL* 遺伝子群は機能的にもあるいは物理的にも相互作用していることが明らかとなった。一方、*SCF* RNAi 変異体では *MSL* 遺伝子群の発現、そのタンパク質の X 染色体への局在、あるいは X 染色体の hyper-acetylation は変化しておらず、*SCF* 遺伝子は *MSL* 遺伝子群の下流で機能していることが示唆された。SCF タンパク質は全染色体にわたって分布しているが、*MSL* 遺伝子群の一つ、*mof* 遺伝子の変異体では SCF タンパク質の X 染色体上の分布が減少している事実もこのことを支持している。さらに、*SCF* 遺伝子を強制発現し唾腺染色体を観察すると、雌の X 染色体のみそのバンドパターンが乱れ、構造がより幅広くなる異常が観察され、SCF がクロマチン構造を実際に変化させうることを示していた。

以上の実験結果は、*in vitro* において DNA に負の超らせんを導入する SCF が *in vivo* においても染色体構造を変化させることにより実際に遺伝子転写活性を調節していることをはじめて示したものであり、非常に興味深いものと言える。このことにより審査委員会は全会一致でこの論文が博士論文として十分であるとの結論に達した。