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論　文　内　容　の　要　旨

〔　題　　名　〕

Molecular mechanisms that link the establishment of chromosome cohesion to DNA replication in *Xenopus* egg extracts

(アフリカツメガエル卵抽出液におけるDNA複製反応と協調した姉妹染色体接着の成立機構)

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Sister chromatid cohesion mediated by the cohesin complex is crucial for accurate segregation of chromosomes. Cohesin is loaded onto chromatin before DNA replication and entraps sister DNAs during DNA replication. Establishment of cohesion requires the cohesin acetyltransferase (CoAT), whose known substrates for cohesion establishment are two conserved lysine residues in the Smc3 cohesin subunit. In vertebrates, which have two CoAT orthologs, CoATs promote chromatin loading of Sororin, an essential cohesin accessory protein that promotes stable chromatin association of cohesin. In yeast, the CoAT activity is required during S-phase to establish cohesion, and Smc3 acetylation increases as cells enter S-phase. CoATs carry a degenerated version of PCNA-interacting peptide (PIP) box, which is essential for cohesion establishment and partially required for chromatin loading of CoAT in yeast. In contrast, vertebrate CoATs have long N-terminal extensions that are reported to bind to chromatin, and how CoATs are loaded onto chromatin and when Smc3 is acetylated are not well studied in vertebrates. How two vertebrate CoATs are involved in cohesion establishment and why two CoATs are present in vertebrates are also not well understood. Moreover, whether Smc3 is the only essential target of CoATs remains elusive. Most importantly, how CoAT function is coupled to the process of DNA replication has still been unclear in any organisms.

To understand the mechanism of cohesion establishment during DNA replication, I analyzed regulation and function of vertebrate CoATs using *Xenopus* egg extracts, which is the only model system that recapitulate cohesion establishment coupled with DNA replication *in vitro*. In *Xenopus*, two CoAT orthologs, XEco1 and XEco2, have already been identified. I found that XEco2 is a predominant CoAT in *Xenopus* egg extracts. XEco1 was not detectable in eggs and in early embryos, but became detectable after developmental stages where the cell cycle acquires somatic characteristics. XEco2 but not XEco1 was responsible for Smc3 acetylation and cohesion establishment in the egg extracts. Smc3 acetylation was dependent on the assembly of the pre-replication complexes (pre-RCs) that promote chromatin binding of cohesin, but was independent of not the initiation of DNA replication. Consistently, XEco2 loading was independent of the initiation of DNA replication, and required pre-RCs. The pre-RC-dependent XEco2 loading was not dependent on cohesin loading, and also XEco2 was not required for cohesin loading, indicating that pre-RCs recruit XEco2 and cohesin onto chromatin through independent pathways. In the N-terminal extension of XEco2, I identified two motifs, which I named as the motif-A and B, required for its pre-RC-dependent chromatin binding. The motif-A and B were required for Smc3 acetylation, Sororin recruitment and cohesion, but were not required for acetyltransferase activity of XEco2. These data suggest that the pre-RC-dependent XEco2 loading is essential for cohesion establishment in *Xenopus* egg extracts. I also found that, although Smc3 is acetylated before the initiation of DNA replication, interaction of acetylated cohesins with DNA becomes more stable after DNA replication, suggesting that in addition to pre-replicative Smc3 acetylation, a replication-coupled reaction, possibly Sororin loading, is required for cohesin stabilization in S-phase. Interestingly, I also found that the 'PIP box' and a short motif nearby the PIP box are required for Sororin recruitment but dispensable for Smc3 acetylation. Consistently, to promote loading of Sororin, XEco2 activity was still required even after pre-RC-dependent Smc3 acetylation is carried out, suggesting that XEco2 might acetylate substrate(s) other than Smc3 to induce Sororin loading during DNA replication. Collectively, my data revealed, for the first time, that the process of DNA replication regulates the CoAT-dependent cohesion establishment reaction at multiple stages during G1 and S-phases of the cell cycle.

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

細胞が分裂して増殖する過程において、遺伝子情報を担う染色体DNAを正確に１回だけ複製し娘細胞に均等に分配することが生命の継承に必須である。コヒーシンタンパク質複合体による姉妹染色体接着は、M期での姉妹染色体の正確な分配を保証ために、真核生物に広く保存される重要なしくみである。コヒーシンはDNA複製前に染色体に結合し、DNA複製と協調して姉妹染色体接着を成立するが、その仕組みは十分に理解されていない。

申請者は、染色体研究に適したモデル系であるアフリカツメガエル卵無細胞系を用い、姉妹染色体の接着に必要なコヒーシンアセチル基転移酵素(CoAT)によるコヒーシンSmc3サブユニットのアセチル化機構を明らかにした。脊椎動物ではEco1,Eco2の2つのCoATホモログが存在する。ツメガエル卵抽出液ではXEco2が姉妹染色体接着を担うことを明らかにした。さらにXEco2は、G1期染色体上に形成される複製前複合体（pre-RC）に依存して染色体に結合し、複製開始前にSmc3をアセチル化することを明らかにした。XEco2の染色体結合に必要なN末端領域の保存されたモチーフを同定し、pre-RC形成と協調したXEco2の染色体結合が姉妹染色体接着の成立に必須であることを示した。さらにXEco2は複製反応と協調してコヒーシンの安定化に必要であり、この反応にはXEco2のC末領域の保存されたモチーフが関与することを見いだした。本研究結果より、脊椎動物CoATの機能がDNA複製過程の特定の反応と密接に協調することが初めて明らかになった。

これらの結果はきわめて新規性が高く、真核生物のゲノム維持機構を理解する上で重要性がある。これらの結果を学位論文「アフリカツメガエル卵抽出液におけるDNA複製反応と協調した姉妹染色体接着の成立機構」としてまとめた。よって、本論文は博士（理学）の学位論文として十分に価値があるものと認める。