論文の内容の要旨

論文題目： Genetic and biochemical analyses of mammalian Claspin, a factor required for genome replication
（動物細胞複製因子 **Claspin** の機能の遺伝学的および生化学的解析）

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Claspin is an important upstream regulator of the checkpoint factor Chk1, originally discovered in Xenopus and conserved from yeast (mrc1) to human. Claspin plays a critical role as a mediator molecule that transmits the replication stress signal from ATR to Chk1 effector kinase. Claspin binds to Chk1 via a region known as the Chk1-binding domain (CKBD) that contains multiple phosphopeptide motifs that are phosphorylated by an unknown kinase and are needed for ATR-dependent phosphorylation of Chk1. Cdc7-ASK has been reported to bind and phosphorylate Claspin. In addition to its well-known important role for initiation of DNA replication at each origin, Cdc7 plays a role also in replication stress checkpoint. However, the details on the roles of Cdc7-Claspin interaction during normal replication and replication stress response have been unclear. Therefore, we generated Claspin-deficient mice and established Claspin conditional knockout cell lines to investigate their functions.

The Claspin-deficient embryos are dead between E9.5 and E12.5. Conditional knockout of Claspin in MEF cells resulted in deficient replication checkpoint and reduced DNA replication. I have demonstrated the important role of the acidic patch near the C-terminal of Claspin during normal DNA replication as well as during checkpoint response. The alanine substitution of the acidic residues (DE/A) in the acidic patch led to loss of binding to Claspin of important initiation factors including Cdc7, Cdc45, DNA polymerase ε/δ, and TopBP1. Purified Cdc7 protein physically interacts with Claspin, but this binding is lost by the DE/A mutation. The DE/A mutant cannot be phosphorylated by Cdc7-ASK in vitro, either. Importantly, phosphorylation of Mcm2 and 4, critical targets of Cdc7 and essential for replication, is greatly reduced by the DE/A mutation of Claspin, indicating that Claspin plays an important role in recruitment of Cdc7 to pre-replicative complexes. I also show that the acidic patch can interact with the N-terminal segment of Claspin and reduces DNA binding activity of the N-terminal segment as well as PCNA binding to Claspin (through the newly identified PIP box present in the N-terminal

segment). DE/A mutation and an internal deletion of the acidic patch resulted in much stronger DNA binding activity, consistent with the above finding that the acidic patch can inhibit the DNA binding of the N-terminal segment. These results show a crucial and novel role of Claspin in initiation of DNA replication through recruiting an essential kinase. The results also point to an intramolecular interaction as a potentially conserved and important mean for regulation of protein functions.

After release from serum starvation, MEF cells lacking Claspin cannot progress cell cycle normally and cells are dead by 48 hours after the release. During release, p53 is induced but MCM2 is absent in the Claspin mutant. The DE/A mutant is defective in this process, but the PIP mutant Claspin (unable to bind to PCNA) is proficient. These results imply a critical role of Claspin during the recovery from the quiescent (resting) state.