論文の内容の要旨

Role of $p27^{Kip1}$ phosphorylation in Cdc6-driven activation of p27-bound Cdk2.

(Cdc6 による$p27^{Kip1}$ 結合Cdk2 の活性化におけるp27 のリン酸化の役割)

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During the G_1-S transition, $p27^{KIP1}$ a potent inhibitor of Cyclin Dependent Kinases inhibit the kinase activity of Cdk2. This $p27^{KIP1}$ in rodent fibroblasts undergoes post-translational modification by phosphorylation, at serine-10, threonine-187 and C-terminal threonine-197 sites by KIS, Cdk2, and Pim or ROCK kinases, respectively. Recently Cdc6 the AAA+ ATPase, identified initially to assemble pre-replicative complexes on origins of replication and later to activate $p21^{CIP1}$-inactivated Cdk2, was also found to activate p27-bound Cdk2, but only after the bound p27 is C-terminally phosphorylated at threonine-197. On the other hand, the biological significance of the serine-10 phosphorylation remains elusive, beside its involvement in p27's stability.

I report here that serine-10 phosphorylation is pre-requisite for efficient C-terminal

phosphorylation of itself by Pim or ROCK kinases, and also critically controls the potency of p27 as a Cdk2 inhibitor. In, *In-vitro*, Pim1 and active ROCK1 can efficiently phosphorylate free as well as Cdk2-bound p27, but only after the p27 was phosphorylated at serine-10 in advance. Consistently, an S10-nonphosphorylatable mutant of p27 protein could not be phosphorylated at the C-terminus *in vivo*. Furthermore, when unbound form of p27 was doubly phosphorylated, it could no longer act as a potent inhibitor of Cdk2, whereas Cdk2-bound p27 could be removed by Cdc6 to reactivate the Cdk2 only after it get phosphorylated at both sites. Thus, phosphorylation at these two sites crucially controls the potency of this CDK inhibitor in two distinct modes.