

## 論文の内容の要旨

Role of Mammalian Target of Rapamycin Complex 1 pathway and Extracellular Signal Regulated Kinase pathway in regulating the activating phosphorylation of Cdk4 and Cdk6.

(Cdk4 および Cdk6 の活性化のためのリン酸化の制御における mTORC1 および ERK シグナル経路の役割)

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The cdks play a crucial role in progression of the cell cycle through G1-S phase. Fibroblast cells upon anchorage deprivation get arrested at G1 phase of the cell cycle leading to inactivation of cdks and destabilization of Cdc6 protein. According to findings in our laboratory, anchorage deprivation from rat embryonic fibroblasts (REF) leads to inactivation of the mTORC1 pathway, whereas in REF cells with constitutive activation of mTORC1 all the effects of anchorage deprivation are overcome. It was also observed that inhibition of the mTORC1 pathway by rapamycin reduces the activity of Cdk4 kinase. Therefore to find the link between mTORC1 pathway and the regulation of activating phosphorylation of Cdk4, two-dimensional electrophoresis was used as a tool. By constructing and comparing Thr172Ala and Thr177Ala non-phosphorylatable mutants with wild type Cdk4 and Cdk6 respectively, I supposed that Thr172- Cdk4 and Thr177- Cdk6 are represented by the most negatively charged spot no.4 in isoelectric focusing dot patterns. In *Tsc2<sup>-/-</sup>* REF with

overexpression of Cdc6 and Rheb proteins, I found that inactivation of both mTORC1 and ERK pathways are required for suppression of activating phosphorylation of Cdk4/6. I also found that S6 kinase inhibition and ERK inhibition also leads to a complete disappearance of Thr172/177 Cdk4/6 phosphorylation. I further found that inhibition of both mTORC1 and ERK inhibits the assembly of Cdk7 with Cyclin H. All these data suggest that either one of the mTORC1 or ERK pathways are required for the formation of CAK (Cyclin Activating Kinase). Consistently, Mat1 the assembly factor for CAK contains a consensus S6kinase phosphorylation-target like sequence conserved among different organisms for example human, mouse, rat, xenopus, and drosophila.

