論文内容の要旨

論文題目　FUNCTIONAL DISSECTION OF MAMMALIAN CDC7 KINASE

（ヒト Cdc7 キナーゼの機能部位の同定）

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Cdc7 is a serine/threonine kinase conserved from yeasts to human. Its catalytic function during cell cycle is activated via association with the activation subunit, Dbf4/ASK, through conserved Dbf4-motif-M and Dbf4-motif-C. However, ASK-interacting motif in Cdc7 remained undefined. On the other hand, since major targets of Cdc7 during initiation of DNA replication are present on the chromatin, association of Cdc7-ASK with chromatin may increase as S phase initiates and proceeds. In order to map the potential chromatin- and ASK-interacting domains in Cdc7 kinase, we generated a series of Cdc7 truncation mutants and cloned them into a CSII-EF-MCS lentiviral vector carrying both HA- and mKO2 (monomeric Kusabira Orange 2)-fluorescent tags. Each plasmid was then used for transfection into 293T followed by transduction into HeLa cells. Subcellular localization and oscillation in the protein level of each mutant were examined via time-lapse analyses. Chromatin binding was assayed by fractionation of the cells expressing each mutant. Association between Cdc7 (HA-tagged) and ASK (FLAG-tagged) was examined by transient coexpression of both plasmids into 293T and HeLa cells followed by coimmunoprecipitation assays. Our results indicate that the segment near the C-terminus of Cdc7, spanning the kinase insert III (amino acids 440-538), is essential for association with ASK. This segment, along with other segments, apprears to be required for chromatin binding of Cdc7. UV photocrosslink assay as a different approach revealed that 444Gly of Cdc7 is very close to ASK in Cdc7-ASK complex. The level of ectopically expressed Cdc7 fusion protein decreased during G1 phase, strongly suggesting that the protein may be regulated by cell cycle-specific proteolysis.