# 論文審査の結果の要旨

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This thesis comprises of four (4) chapters: (I) Introduction, (II) Results, (III) Discussion and Conclusion, and (IV) Materials and Methods. In the first chapter, background on DNA replication and functions of Cdc7 reported to date are elaborated. In the second chapter, novel functions of human Cdc7 during G2 and M phases of the mammalian cell cycle are discussed. The third chapter includes the general discussion and concluding remarks, while the forth chapter covers the research methodologies related to this work.

Cdc7 (cell division cycle 7), an evolutionary conserved serine/threonine kinase, is an important DNA replication regulatory protein overexpressed in many human cancers and neoplastic cells. Studies demonstrating selective induction of cell death in the human cancer cells but not in the normal fibroblasts after Cdc7 inhibition have highlighted Cdc7 as a useful candidate target for cancer therapy. Nevertheless, limited knowledge on the biological functions of human Cdc7 outside S phase may pose limitations to the potential and specificity of the Cdc7 inhibitors as anti-cancer drugs. In this work, Toh Gaik Theng （トウ　ゲック　ティング）describes the novel roles of human Cdc7 as an important regulator during G2 and M phase progression through evaluations of the physical and/or functional interactions between Cdc7 and mitotic kinases such as Plk1 and Aurora B.

Section 2.1 and 2.2 of this thesis show the oscillation pattern of human Cdc7 protein during cell cycle through western blot analysis and time-lapse imaging Cdc7-fusion expressing cells. Cdc7 is suggested as an unstable protein which undergoes degradation at mitotic exit. Section 2.3 and 2.4 describe Cdc7 as target of APC/$C^{Cdh1}$-mediated proteolysis which carries a putative degradation box (D-box) in its C-terminal. Section 2.5 and 2.6 demonstrate chromatin localization of Cdc7 in Mitotic (M) phase and the effects of siRNA-mediated Cdc7 depletion on cell cycle progression, particularly on that of G2- and M-phase. Delayed G2 phase progression and inefficient mitotic exit (i.e. M/G1 transition) observed in the Cdc7-depleted cells implicate the involvement of Cdc7 in regulating these cell cycle phases. Consistent with this, section 2.7 shows identification of the Plk1 mitotic kinase as a Cdc7 interacting protein during M phase. Section 2.8 and 2.9 further discuss about the functional significance of the Cdc7 and Plk1 interaction *in vivo*. Simultaneous expression of Cdc7, Dbf4/ASK (a Cdc7

regulator) and Plk1 alleviates G2/M phase arrest caused by Plk1 overexpression, plausibly by improving M/G1 transition since interaction between Plk1 and Cdt1 DNA licensing factor is weakened in these cells. Cdc7 is suggested to antagonize interaction between Plk1 and Cdt1 via N-terminus of Plk1, thereby releasing Cdt1 necessary for efficient pre-replicative complex formation during DNA origin licensing at M/G1 transition. Stimulation of Aurora B kinase activity by Cdc7 *in vitro* which implies a potential crosstalk between Cdc7 and multiple mitotic kinases during cell cycle regulation is also discussed. Finally, section 2.10 confirms that depletion of Cdc7 by siRNA reduces Cdt1 chromatin loading and accumulation of G2/M phase population, similar to that observed in the Cdt1-depleted cells. Based on these findings, a mechanism by which Cdc7 regulates DNA origin licensing through interaction with Plk1 is proposed, unraveling novel roles of Cdc7 during cell cycle regulation.

With the abovementioned, I hereby acknowledge that the scope and quality of this thesis is qualified for the award of the degree of Doctor of Philosophy in Life Sciences.

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