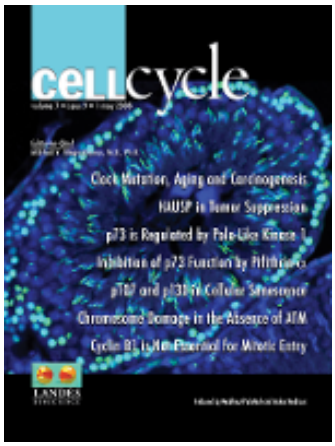

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Letter to the Editor

Cdc25A promotes G2/M transition in oocytes

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The mammalian cell cycle is controlled by a cluster of protein kinases and protein phosphatases, among which Cdc25 phosphatases have been evidenced to remove the inhibitory phosphorylation of CDKs to activate the kinases and thus promote mitotic progression.[1,2] In mammals, the Cdc25 family includes three homologs, Cdc25A, B and C.[3] Cdc25B and C are believed to play crucial roles in G_2/M progression,[4,5] whereas Cdc25A has long been thought to function only in G_1/S progression.[6,7] Nevertheless, recent studies revealed the novel roles of Cdc25A through G_2 and mitosis phase,[8,9] and Cdc25a$^{-/-}$ mouse showed early lethal embryos,[10] indicating its new potential during the cell cycle. However, the contribution of Cdc25A to meiotic resumption is still unclear.

Oocytes are arrested at the diplotene stage of the first meiotic prophase, which is termed germinal vesicle (GV) stage. In vitro, fully-grown immature oocytes restart their meiotic process when removed from the inhibitory environment of follicles, indicated typically by germinal vesicle breakdown (GVBD). In this study, we investigated the role of Cdc25A in G_2/M transition by overexpression of this protein and showed that Cdc25A is a new promoter for GVBD during meiotic resumption in mammals.

Total RNA was extracted from 100 mouse GV oocytes and the first strand cDNA was generated with cDNA synthesis kit (Takara) by using poly (dT) primers. The following two nested primers were used to clone the full length of Cdc25A cDNA by PCR. F1: GAAGACGGAGCGGGAGT, R1: CTGGGAGTGTAGGAGACAGG, F2: TCAGGCCGGCCGATGGAACTGGGCCCGGAG, R2: GTTGGCGCGCCTCAGAGCTTCTTCAGGCG. For in vitro transcription reactions, the Cdc25A cDNA was subcloned into the modified pCS2$^+$ vector, which has NH_2-terminal Myc_6. The Myc-Cdc25A-pCS2$^+$ plasmid was linearized by SalI and purified by gel extraction kit (Tiangen). SP6 message machine (Ambiom) was used for producing capped mRNA which was purified using the RNeasy cleanup kit (Qiagen). The concentration was detected by DU 530 analysis, and 2.5 mg/ml Myc-Cdc25A mRNA solution was injected into the cytoplasm of GV stage oocytes. Microinjections were performed using an Eppendorf microinjector and completed within 30 minutes. To prevent the spontaneous maturation of mouse oocytes in vitro, 2 μM milrinone was used to keep the oocytes at GV stage. The same amount of H_2O or Myc_6 mRNA (virtually no discrepancy was obtained) was injected as control.

We firstly examined the localization of the overexpressed Cdc25A in GV stage oocytes. As represented in Figure 1A, the tagged protein was obviously localized in the GV, and little staining was observed in the cytoplasm, consistent with the previous reports showing that Cdc25A is mainly nuclearly localized.[7] To determine whether this protein plays a role as a promoter in G_2/M transition, we examined the GVBD rates during the culture. As shown in Figure 2, no oocyte underwent GVBD in Myc mRNA injected (control) group. However, in Myc-Cdc25A mRNA-injected group, 30% oocytes could overcome the milrinone inhibition and completed GVBD, confirming that the overexpressed Cdc25A exerted a stimulative role in G_2/M transition. Indeed, not all the oocytes successfully overexpressed the injected Cdc25A mRNA. By testing the oocytes under the confocal microscope, we found that approximately 40% of them presented the positive signal, meaning that a considerable number of oocytes failed to express the exogenous protein. In oocytes overexpressing Cdc25A, the GVBD rate should be even higher than that observed, i.e., 30%/40% = 75%, indicating the more effective contribution of Cdc25A to the G_2/M transition.

Among the GV oocytes with overexpressed protein, we noted that some of them had positive staining on nuclear envelop rather than in the GV (Fig. 1B), indicating possible migration of Cdc25A during the transition. To further address this issue, we tested the localization of Cdc25A in oocytes at different phases.

Interestingly, in the oocytes at GVBD stage, the signal in some of them exhibited a regular ring with a diameter bigger than the GVBD area (Fig. 1C). This result appears to link a recent work suggesting that, with the N-terminal nuclear export sequence and nuclear localization signal, Cdc25A shuttles between the nucleus and cytoplasm,[11] which implies the multiple roles of Cdc25A in the cell cycle regulation in addition to its main function in the nucleus. Moreover, considering the reported behavior of active Cdc2-cyclinB complex accumulating in the nucleus where it phosphorylates multiple targets to cause nuclear envelop breakdown[12,13] and the interaction between this complex and Cdc25A through the cell cycles,[8,14] we speculate that the behavior of Cdc25A during G_2/M transition is closely related to Cdc2-cyclinB, which mediates GVBD. But the detailed mechanism needs further investigation.

Nevertheless, by testing the oocytes at the first prometaphase (Pro-MI), we found that the regular ring disappeared (Fig. 1D). When these oocytes reached first metaphase (MI), the protein assembled at the spindle, but its signal was much weaker than that in the GV (Fig. 1E). Subsequently, no staining of the protein could be detected in the MII-oocytes (second metaphase) (Fig. 1F). These data implied an degradation of Cdc25A in the G_2/M transition (at the GVBD point), similar to a pervious observation that Cdc25A level rapidly decreased as HeLa cells were released into the cell cycle from a nocodazole-induced arrest.[14] Alternatively, it is possible that the exogenous Cdc25A is not stable enough to stay for a longer time without adequately activated Cdk1 (if any) within the oocyte, because Cdc2-cyclinB-dependent phosphorylation stabilizes Cdc25A, thereby further increasing the cellular phosphatase potential to keep Cdc2 in its active form. i.e., a feedback loop forms between Cdc2 and Cdc25A.[8] However, this hypothesis needs to be further evidenced.

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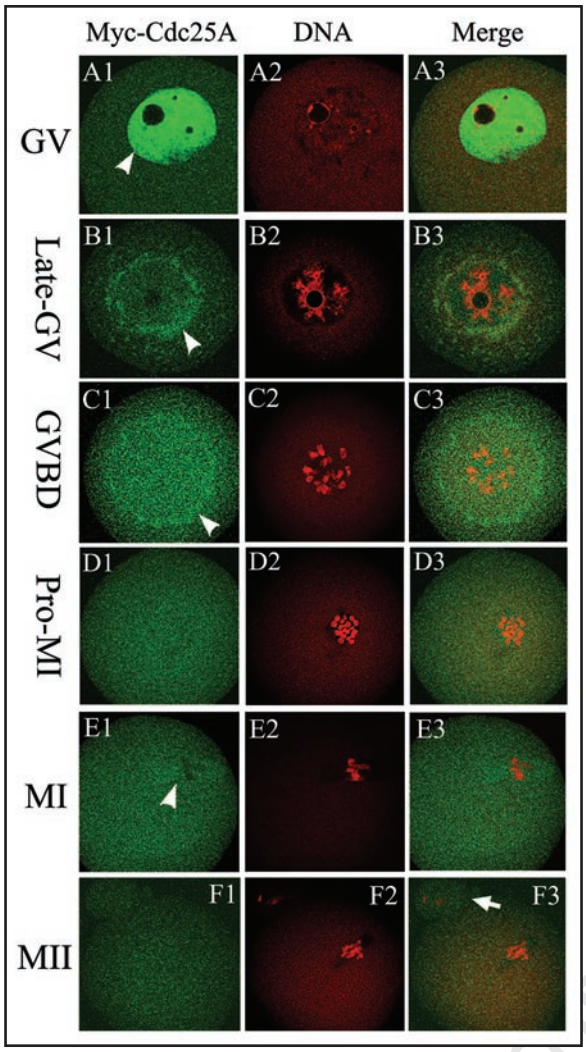

Figure 1. Localization of overexpressed Cdc25A during meiotic maturation. (A) Strong signal was observed in the nucleus in GV-oocytes; (B) In late GV-oocytes (shortly before GVBD), the protein translocated to the area around the nuclear envelop; (C) In GVBD-oocytes, the signal was in a ring, which was a little bigger than the original nuclear area; (D) Dispersed signal or no detectable signal was observed in Pro-MI oocytes; (E) Weak signal was observed in MI-oocytes, which was associated with the spindle; (F) Dispersed signal or no signal could be detected in MII-oocytes. Arrowheads indicate the signal of overexpressed Cdc25A and arrows indicate the polar body.

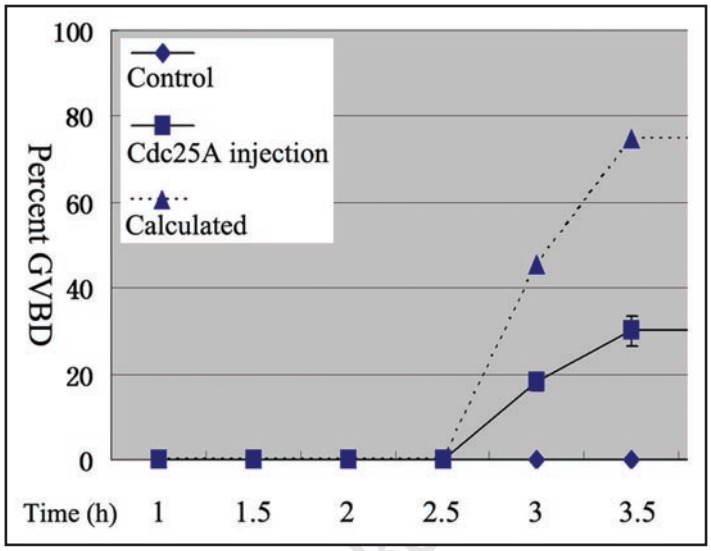

Figure 2. Effect of overexpressed Cdc25A on GVBD of oocytes. Oocytes were cultured in M16 in the presence of 2 μM milrinone. Each group included at least 50 oocytes. During the culture, oocytes were counted for GVBD at different time points. Control group: oocytes were injected with 2.5 mg/ml Myc mRNA. No oocyte underwent GVBD during the culture; Cdc25A injection group: oocytes were injected with 2.5 mg/ml Myc-Cdc25A mRNA. Before 2.5 hour, no oocytes could undergo GVBD. At 3 hour, a few of the oocytes completed GVBD (18.2%) and the percentage reached the maximum of 30% at 3.5 h and kept stable for the subsequent time. Calculated group: oocytes were injected with 2.5 mg/ml Myc-Cdc25A mRNA and cultured for 2 hours to allow the protein expression followed by examination of the overexpressed signal under confocal microscope (Because in this study, oocytes showed the strongest stain at approximately 2 hours during the culture). Approximately 40% oocytes exhibited the positive signal. Therefore, the GVBD percentage in Cdc25A overespressed oocytes should be 75%.

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