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Effects of Protein Kinase A- and C-mediated Pathway on Maturation Promoting Factor Activity during G₂/M Transition in Mouse Fibroblast LP1-1 Cells

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Maturation promoting factor(MPF) is an almost universal concomitant of M phase, and is responsible for catalyzing the G₂/M transition in all eukaryotic cells. But the regulatory mechanism on MPF activity during G₂/M transition is still unclear. In this report, whether MPF activity can be regulated by the PKA- or PKC-mediated pathway was examined in mouse fibroblast LP1-1 cells. In order to investigate MPF activity during G₂/M transition, LP1-1 cells were synchronized at G₁/S boundary and then the levels of cyclin B1 and cdc2 mRNA were determined at each phase of cell cycle. MPF activity was determined at the same time points. Expression and MPF activity were high during G₂ phase and decreased at a time corresponding to G₁ phase. After treatment of cyclic AMP analogues (Dibutyryl cAMP, 8-bromo-cAMP), a PKA stimulator, MPF activity was decreased at G₂ phase in time- and dose-dependent manner. However, PMA(Phorbol 12-Myristate 13-Acetate), a PKC stimulator and calphostin C, a PKC inhibitor, have no effect on MPF activity at G₂ phase in LP1-1 cells. These results suggest that PKA-mediated pathway has an effect on MPF activity during G₂/M transition in LP1-1 cells as compared with PKC-mediated pathway.

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Effects of Cyclic AMP on D-type Cyclins Expression during G₁ Phase in Mouse Fibroblast LP1-1 cells.

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D-type cyclins are likely to play a key role in controlling cell cycle progression at G₁ phase, although their regulatory mechanism during G₁ phase in higher eukaryotic cells is unclear. In order to investigate the effect of cAMP on D-type cyclins expression during G₁ phase, LP1-1 cells were synchronized at G₁ phase by serum starvation for 48 hours and then the levels of cyclin D1 and cyclin D3 mRNA were determined at each phase of cell cycle. Expression of cyclin D1 gene was high during G₁ phase and decreased at S phase, while cyclin D3 gene expression was high during late G₁ and S phase. After treatment of cAMP analogues(Dibutyryl cAMP, 8-bromo cAMP) in LP1-1 cells, cyclin D1 and D3 gene expression was decreased at G₁ phase comparing to that of the control. Furthermore, DNA synthesis rate was decreased at S phase after treatment of cAMP analogues in LP1-1 cells. These results suggest that cAMP have an inhibitory effect on D-type cyclins expression during G₁ phase and G₁/S progression in LP1-1 cells.