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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.