F801 Expression of *Saccharomyces cerevisiae CDC6* gene in insect Sf9 cells and analysis of its interaction with MCM proteins

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Cdc6 protein (Cdc6p) plays a crucial role in the regulation of replication initiation in all eukaryotes. Cdc6p is synthesized at the end of mitosis, and allows the formation of prereplicative complex. Cdc6p binds to the chromosomally bound Origin Recognition Complex(ORC) at this time, and enables the subsequent binding of Minichromosome Maintenance (MCM) proteins at the replication origins. The prereplicative complexes are fired when the S-phase cell cycle kinases Clb5,6/Cdc28p are activated in late G1. It is not known which proteins are physically interacted in the prereplicative complexes. In this study, polyhistidine tagged and untagged Cdc6p have been cloned and expressed in insect cells. Polyhistidine tagged Cdc6p was purified by a single step using Ni-NTA agarose. The purified protein was reacted with antibodies against polyhisdine as well as Cdc6p, indicating that the purified protein is Cdc6p. In order to study interaction of Cdc6p with MCM proteins, recomibinant baculovirus expressing Cdc6p was coinfected with each of recombinant viruses expressing MCM2, 3, 4, 5, 6, and 7. Western blot analysis of MCM proteins copurifed with poyhistidine-tagged Cdc6p by Ni-NTA agarose indicate that Cdc6p was not phsically associated with any of the MCM proteins under the present conditions.

F802 Analysis of Transcriptional Regulatory Region of Hermaphroditic Fish rmHSC 71(*Rivulus marmoratus* heat shock cognate protain 71) Gene

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Fish have been recently spotlighted as a model for the vertebrate developmental genetics, because they have lots of genetic merits such as short generation time, easy to raise as an experimental animal, genetically easy to manipulate due to *in vitro* fertilization and transparent egg, the simple genomic contents, etc. However, in spite of the prominent progress in the research related to gene regulation, transcription regulatory mechanism of fish has not much known. To get more insight on gene regulation of fish, we started to analyze the transcriptional regulation of *R. marmoratus* heat shock cognate protein 71 (rmHSC 71) gene. rmHSC 71 belongs to heat shock protein family and shows tissue tropism in expression such that the strongest expression appeared in muscle and brain. To investigate transcription regulatory region of rmHSC 71, we identified at first a transcription start site and an untranslated exon I by primer extension and cDNA sequencing. Exon I is separated from exon II by 690 bp and the length of exon I is 67 bp. TATA box is located at 30 bp upsteam from the 5′ end of exon I. Next, we made 10 serial deletion mutants, containing variable sizes of 5′ flanking region of rmHSC 71 gene and then linked them to the reporter, enhanced green fluorescence protein (EGFP) gene. Levels of expression of each constructs were assayed by RT-PCR assay or by counting the EGFP expressing cells in fluorescence microscope at 48 hr after transient transfection of constructs into the monkey-kidney cell line (E25B2) or fish liver cell line (PLHC). The transcriptional activities in the region between -2.6 Kb and -0.7 Kb show some discrepancies in between E25B2 and PLHC cells, while the region between -0.2 Kb and a transcription start site shows a positive effect in both cell lines. These data suggest two facts; 1) similar but different factors are involved in the transcription of mammals and fish and 2) a minimal promotor of rmHSC71 is localized within 200 bp from the transcription start site. Currently we are investigating the protein-DNA interactions in this region by gel shift assay.