D103 Functional Role of Transcription Factor CP2 on the Cell Fate Determination during Early Development

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We recently revealed that the increment of CP2 activity during induced differentiation of MEL cells is critical for the expression of globin genes. On the other hand, CP2 is not restricted its expression on erythroid lineages and rather it also expressed in several culture cell lines derived from variety of tissue types. Furthermore, CP2 expression was observed in almost every tissues examined during development and differentiation, even though the levels of expression were variable among tissues. Because CP2 starts to appear in hatched morula, it may speculate that CP2 plays essential role during early stage of development. In order to define the functional role of CP2 in early development, we introduced antisense CP2 expression construct into ES cells to reduce the steady state level of CP2, and then examined the expression profile of several lineage marker genes in differentiating embryonic bodies *in vitro* as well as cell type determination in teratomas formed in nude mice by subcutaneous injection of ES cells. Interestingly enough, any cell type originated from mesoderm was not observed at all while those of neuroectoderm were differentiated much faster than normal. These results suggest that transcription factor CP2 may be involved in earlier stages of cell fate decisions.

D104 Calsequestrin homologue of *C.elegans* is expressed in body-wall muscle

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Calsequestrin is one of the major calcium binding proteins localized in sarcoplasmic reticulum (SR) of skeletal and cardiac muscles of vertebrates. Its biochemical properties, a high capacity and a low affinity of calcium binding, suggested that it may function as a calcium buffer in SR. The database of *C.elegans* genome project revealed a calsequestrin-like sequence which exhibits 50% similarity with rabbit skeletal calsequestrin in amino acid level. We are interested in studying this putative homologue of calsequestrin in *C.elegans* in order to elucidate its function in muscle cells.

We have cloned and sequenced cDNA encoding *C. elegans* calsequestrin. Full-length open reading frame except the signal peptide and C-terminal half of calsequestrin were expressed in *E.coli*. Both forms of calsequestrin were found to bind $^{45}Ca^{2+}$ in a ligand overlay. Northern blot analysis showed a transcript of 1.45kb throughout developmental stages. A single protein, which migrates at about 64kDa, was recognized by polyclonal antibody. Immuno-staining showed striated patterns in boddy-wall muscle cells. Calsequestrin promoter / GFP (green fluorescence protein) fusion gene was expressed in body-wall muscle of transgenic worm. This pattern is consistent with immunostaining data. Results presented here show that we have cloned a calsequestrin of *C. elegans*, which has a calcium binding activity.