

ABSTRACT

THESIS: Regulation of Expression of the Liver, Muscle, and
Brain/Platelet/Placenta Isoforms of Phosphofructokinase in
Preimplantation Mouse Embryos

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Glycolysis is one of two metabolic pathways in which most mammalian cells generate energy. Preimplantation mouse embryos do not run the glycolysis pathway until the 8-16 cell stage. A major regulator of glycolysis is the enzyme 6-phosphofructo-1-kinase (PFK). Jeff Henry's research found that mRNA for the liver specific form of PFK is found inconsistently at the 1-cell stage and consistently from the 2-cell stage through the blastocyst stage and that mRNAs for muscle or brain/platelet/placenta isoforms of PFK were not expressed at any stage. Studies suggest that PFK is not enzymatically active in the mouse embryo until the 8-cell stage when glycolysis begins. Using specific polyclonal anti-PFK antibodies, immunofluorescence and laser confocal microscopy, we found that the liver specific PFK protein expression follows the same pattern as the

mRNA expression in preimplantation mouse embryos. The muscle and brain/platelet/placenta PFK protein antibodies stained embryos in the absence of mRNA but the expression pattern did not follow the enzyme activity pattern with results that suggest specificity problems or cross reactivity of the antibodies. The liver specific PFK isoform expression showed an increase in expression throughout development with a significant a increase of 2.3 fold in expression at the 8-16-cell transition. There was also a significant increase of 1.9 fold in PFK-B expression from the 1-cell to the 2-cell stage corresponding to the activation of the embryonic genome. Enzymatic assays are currently being run to look at the activity levels throughout development.