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Cloning and Functional Analysis of Pyruvate Kinase Promoter Region from Drosophila Melanogaster

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Abstract

Pyruvate kinase (PK; EC 2.7.1.40) is a key glycolytic enzyme of Drosophila melanogaster. It catalyzes the conversion of phosphoenolpyruvate into pyruvate with the transfer of a phosphate group to ADP to form ATP. The ATP provides energy for cell growth and metabolism, and pyruvate participates in many metabolic reactions. Therefore, PK plays an important role in cell metabolism. Southern blot analysis, PCR, and sequencing were used to determine the content of a Drosophila pyruvate kinase (Pyk) genomic clone, λPK61. The results indicated that the insert of λPK61 comprised 8330 bp upstream of and 7186 bp downstream of the transcription start point of the Pyk gene. The size of the insert was 15,516 bp in total, which contained six genes including Pyk. Deletion mapping was applied to identify the promoter region and cis-acting elements 5′ of PyK. Ten serial deletions produced by PCR were inserted upstream of the reporter gene (LacZ) to form recombinant plasmids, which were then transfected into Drosophila S2 cells. The results revealed that the regions -1475 ~ -1033 and -1033 ~ -534 of the 5′ end of PyK possessed positive regulatory function for Pyk expression; i.e., increased gene expression. There were redundant putative cis-acting elements, including ecdysone response element (EcRE), E74A, and broad complex zinc finger (BRCZ) binding sites. Both E74A and BRCZ belong to the early genes regulated by ecdysone. This result suggested that Pyk might be regulated by ecdysone, directly or indirectly. However, the results of the developmental profile of Pyk expression by Northern blot analysis suggested that the effects of ecdysone on Pyk were repressive, not inductive. In addition, it was found that in these regions, there were many cis-acting elements related to egg and embryo development. Both -258 ~ -254 and -167 ~ -163 contained a CAAT box, and deletion of these regions decreased reporter gene expression. Therefore, it is suggested that both CAAT boxes are functional and that the promoter of Pyk might be located in the region of -258 ~ +109. No TATA box or downstream promoter element were identified around the transcription start site of Pyk. Additionally, PyK might share a regulatory region with an unknown neighboring gene. It was concluded that Pyk has the characteristics of a housekeeping gene.