ANALYSIS OF RAT LIVER PROTEINS BY TWO-DIMENSIONAL GEL ELECTROPHORES

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Understanding of the molecular mechanisms regulating eukaryotic cell growth and division is one of the major endeavours in molecular biology. To achieve this goal, it is essential to know how DNA replication is initiated and regulated. It is clear that the regulation is associated with the activity of certain proteins whose quantity may be changed substantialy at the boundary of the transition of the cell from the G_1 to the S-phase. Topoisomerase II is one of such examples [1].

There are already many examples of the use of such a high resolving method as two-dimensional electrophoresis for the search for regulatory proteins [2,3]. Not only the detection of the proteins but also the disclosure of their function and structure are of interest. A good example of such an approach is the study of PCNA (proliferating cell nuclear antigen). Its level is directly correlated with the proliferative status of cells, moreover, it is a protein activator of DNA polymerase δ [4].

In this work, the problem of searching for regulatory proteins is solved with the use of regenerating rat liver where up to 50% of cells carry out replication. There are no more than 0.01% of such cells in the normal adult rat liver. The proteins of a homogenate, cytosol, nucleus and an extract of the nuclear membrane, obtained from the normal and regenerating rat liver, were analyzed by the method of two-dimensional electrophoresis [5,6]. Of approximately 200 polypeptides detected in the form of spots on the two-dimensional electrophoretic map after staining with Coomassie R-250, substantial quantitative differences were detected in three ones. The content of the total nuclear protein, p38 (38 *kDa*, isopoint pI = 4) increased 6—8-fold in the S-phase, that of another one, p50 (50 *kDa*, pI = 6.5) dropped by a factor of 2—3. There was also a 2—3-fold decrease in the amount of cytoplasmic protein p35 (35 *kDa*, pI = 8). Moreover, the nuclear protein p40

(40 *kDa*, pI = 6) was detected, its amount increased sharply (up to 20-fold) after a sham operation.

The nuclear protein p38, as a possible regularor of DNA replication, has been purified and its amino acids sequence at N-terminus was determined: ?—L—I—V—F—P—L—N—T. In order to get pure protein, a gel after the electrophoresis was stained by $CuCl_2$, the needed spot was identified, cut out and the polypeptide was eluted. Unfortunately, homology to this sequence in a bank of 10000 proteins was not revealed. Surely, this protein is not PCNA or tropomyosin which possess similar isopoints and molecular masses with p38. Besides that, p38 does not affect DNA polymerase α activity. So its function is not clear as yet. Nevertheless the presented work shows a possibility to use the described approach in the study of pure proteins function and structure after their analysis by two-dimensional gel electrophoresis.

Refences

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