ENZYMATIC COMPOSITION OF REPLICATIVE COMPLEXES FROM RAT LIVER

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The results of the last decade show that DNA replicaton eukaryotic cells is carried out by multienzyme complexes [1]. To understand molecular mechanisms and regulation of their action, it is necessary first of all to study their enzyme and protein composition. We have found a number of DNA polymerase complexes (mol. masses of 300—1500 *kDa*) in nuclear membrane, chromatin,nucleoplasm and cytosol from normal and regenerating rat liver /2—4/. It is considered that an enzyme is a part of replicative complex if 1) the distribution of its activity coincides with the distribution of DNA polymerase activity during initial stages of purification; 2) mol. mass of a purified enzyme is essentially lower than that of the crude enzyme; 3) dissociation and recostitution of a complex are possible. Composition of the complexes has been studied after their purification on the anion-exchanger, salting out and gel-filtration. All the complexes studied contain DNA polymerase α (chromatin complexes contain α and β) and $3' \rightarrow 5'$ exonuclease which may be useful for proofreading of DNA polymerase made errors [5]. However, $3' \rightarrow 5'$ exonucleases can be separated from DNA polymerases by special approaches even in crude extracts, that is DNA polymerases δ and ε have not been found. We have explored in the most detail the largest complex (mol. mass of 1000-1500 *kDa*) located

in nuclear membrane. It manifests activities of primase, helicase, endonuclease (though more than 90 % of this activity is separated in the course of the column chromatography as well as all thymidine kinase), non-specific NTPase, nucleotidase, topoisomerase I, DNA ligase, DNA methylase, protein phosphokinase, and ssb protein. Heavy membrane complex has been found to contain 17—20 major polypeptides, whereas the complex with mol. mass of 400—500 *kDa* has 10—12 polypeptides. Intensity and position of spots (two dimensional electrophoresis in gel) of the nuclear membrane complexes do not change during the cell transition from G_0 to S-period as well as the activity of DNA polymerase α . However, in the complex from chromatic, this activity augments 5—8-fold in the S-period [3]. We have isolated some enzymes from the complexes which copurify in the course of 4—5 stages of the purification (DNA polymerases α [6] and β [7], two $3' \rightarrow 5'$ exonucleases [8], endonuclease [9], protein phosphokinase, and helicase). From 3 to 5% of particles of the heavy membrane complex are connected with DNA (500—1000 base pairs) and RNA (100—130 bases), whereas the other complexes are free of nucleic acids. The heavy complex from the nuclear membrane contains non-transcribed DNA in G_0-period, whereas it is transchribed in S-period. Therefore, DNA is being stretched across the complex in the process of replication [4,10]. So, the replication complexes in question catalyse DNA elongation in vitro and in vivo. The presence of helicase and primase is indicative of their possible participation in the initiation of DNA replication.

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