DNA ELONGATION IN NUCLEI ISOLATED FROM MAMMALIAN CELLS

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Study of molecular mechanisms and regulation of biosynthesis of mammalian DNA is one of the clue problems of modern molecular biology. Application of cell-free systems which are permeable for macromolecules is an advantage in this field. The most native system is isolated crude cellular nuclei. Endogenous DNA synthesis in such nuclei isolated from non-dividing hepatocytes is very small. During transition of the cells from G_0 to the S-period of cell cycle, several-fold acceleration of DNA elongation is observable into the isolated nuclei. However, the use of exogenous DNA as a template and various degree of nuclear injury in the coarse of their isolation results in poorly reproducible data. Different ways of membrane damage of the nuclei isolated from dividing amphibian oocytes lead to repeated initiation of DNA replication in the isolated nuclei [1].

We have shown that the treatment of nuclei (isolated from non-dividing rat hepatocytes) with 1% Triton X-100 in the presence of 20% glycerol is accompanied by the removal of outer nuclear membrane and leads to the activation of endogenous DNA synthesis up to 20—30-fold [2]. The synthesis manifests a linear dependence on nuclear concentration and time of incubation and lasts up to 2 hours. The maximal levels of the synthesis amount to 0.1% as compared with the quantity of pre-existing nuclear DNA. The similar effect is observable with the nuclei isolated from a regenerating liver. Inducible synthesis is proved to be catalysed by DNA polymerase α. It can be either a consequence of replicative initiation or accelerated gap-filling on single-stranded DNA. The analysis of the synthesis products was carried out by several independent approaches: gel-filtration of lyzed nuclei (after synthesis during 0.5—120 *min*) at alkaline conditions; molecular weight distribution of the products of the one-substrate and four-substrates synthesis; the ultracentrifugation of DNA (labelled with Hg^+-dCTP) in density gradient of CsCl; the search for AT-rich products synthesized on the origins of the replication and then melted out at 55°C; γ-irradiation of the nuclei. All the data obtained by these techniques were not in the favour of the replicative initiation. The presence of ssb protein from E. coli, Ap_4A or DNA ligase T_4 in the incubation medium did not influence the DNA synthesis in the purified nuclei. Our Triton treatment did not lead to the augment of the 3'-OH ends quantity in DNA which could lead to the repair synthesis. Presumably we have observed the accelerated DNA elongation catalyzed by DNA polymerase α whose activity is less inhibited by phospholipids of nuclear membrane in the presence of Triton X 100.

So, the nuclei treated with 1% Triton X 100 are the effective system for the measurement of α-polymerase synthesis on the natural mammalian template. Now we have an opportunity to study an action of various preparations of α-polymerase, its complexes and other substances which are unable to penetrate through an intact nuclear membrane. For this purpose, endogenous DNA polymerase α is irreversibly inhibited with 10 *mM* N-ethylmaleimide, then its surplus is neutralyzed with the addition of 20 *mM* 2-mercaptoethanol. Using this approach, we have established that the extracts of nuclear membranecontaining complex forms of DNA polymerase α [3, 4] carry out DNA elongation with an equal effectiveness in G_0 and S-periods of a cell cycle. Therefore, the acceleration of DNA elongation in the replicative period depends not on the change of the properties of α-polymerase complexes bound with nuclear membrane but rather on chromatin loosening at replicative forks.

References

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