POSSIBLE PARTICIPANTS OF "EXTERNAL PROOFREADING" IN MAMMALIAN CELLS

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In eukaryotes, the spontaneous mutation rates have been estimated to be 10^{-10} to 10^{-12} errors per base pair per generation (f.i., [1]). On the other hand, the probabilities of mistakes made by mammalian nuclear purified DNA polymerases are as much as 10^{-4} for α-polymerase and $5 \cdot 10^{-4}$ for β-polymerase (these polymerases lack editing $3' \rightarrow 5'$ exonucleolytic activity), 10^{-5} for δ-polymerase and 10^{-6} for ε-polymerase (these polymerases display "internal" $3' \rightarrow 5'$ exonucleolytic activity, that is their exonuclease active sites are located within polymerase polypeptides) [2]. Moderately processive or distributive DNA polymerases α and β can readily dissociate whenever a misinsertion has taken place. Therefore, base substitutions made by these polymerases could be corrected by "external proofreading" [3] carried out with 1) $3' \rightarrow 5'$ exonucleolytic activity of DNA polymerases δ and ε or/and 2) autonomous $3' \rightarrow 5'$ exonucleases non-associated covalently with DNA polymerases. In the second case, the accuracy of DNA polymerases α from rat liver or calf thymus in the presence of $3' \rightarrow 5'$ exonucleases (reproduction of the primed DNA from phage $\oslash X174$ amber 3) may be augmented by 2–3 orders if *in-vitro* results are extrapolated to the cellular levels of activities of the autonomous $3' \rightarrow 5'$ exonucleases from rat liver nuclei [4].

Relative contributions of the both modes of "external proofreading" to overall replication fidelity can be evaluated by means of comparison of the $3' \rightarrow 5'$ exonucleolytic activities intrinsic to DNA polymerases δ and ε, with those of autonomous exonucleases. It is just what we have explored in the case of rat cell-free extracts.

DNA polymerases δ and ε from regenerating rat liver were partially purified, however their $3' \rightarrow 5'$ exonucleolytic activities and molecular masses were not measured [5]. Autonomous $3' \rightarrow 5'$ exonucleases were shown to be constituents of the mutlienzyme complexes manifesting activities of DNA polymerases α and β in rat liver [4, 6], although the major forms of the purified $3' \rightarrow 5'$ exonucleases had molecular masses of 40–50 kDa [4].

We have used an ability of the complex forms and even the holoenzyme of DNA polymerase α from rat liver to dissociate in the course of ultracentrifugation [7]. In this case, active polymerase subunit has been found in the zone of 156 kDa, and just such mass has been determined for α-polymerase subunit purified to near homogeneity [7]. Consequently ultracentrifugation enables us to separate low molecular autonomous $3' \rightarrow 5'$ exonucleases from $3' \rightarrow 5'$ exonucleases associated covalently with DNA polymerases δ (120–150 kDa) and ε (200–260 kDa) (e.g., [2]), without preliminary fractionation of cell-free extracts and thereby without inevitable and unequal losses of the enzymes under study. Data presented in Fig. show clearly that most of $3' \rightarrow 5'$ exonucleases activity is found in a zone of the proteins with molecular mass of 30–50 kDa, and just this mass is inherent in purified autonomous $3' \rightarrow 5'$ exonucleases from rat liver [4]. Low level of excision from randomly radiolabelled DNA ($\leq 5\%$) throughout the sedimentogrammes proves that we deal with $3' \rightarrow 5'$ exonuclease activity not distorted appreciably with endonuclease activity. Indeed, an activity of the partially purified major hepatic endonuclease displays the inverse ratio (1.7-times in favour of randomly labelled ssDNA as compared with 3'-end labelled ssDNA-substrate) [4]. The left peak of DNA polymerase activity is located in the zone of 100–240 kDa, and it is expected for DNA polymerases α, δ and ε. The application of the either poly[d(A-T)] or oligo(dT)-

poly(dA) instead of activated DNA-template results in decreasing DNA polymerase activity but does not change the position of polymerase peaks. Polymerase activity of the left peak is almost completely inhibited by both N-ethylmaleimide and aphidicolin (the well known inhibitors of DNA polymerases α, δ and ε) whereas polymerase activity of the right peak is insensitive to these inhibitors. Taking into account location of the right polymerase peak in the zone of about 40 kDa too, one may arrive at conclusion that this is activity of DNA polymerase β. In addition, we have never observed the peaks of $3' \rightarrow 5'$ exonuclease activity in the zone of high molecular DNA polymerases. Perhaps the potent activity of autonomous $3' \rightarrow 5'$ exonucleases masks the slight exonuclease activities of minor DNA polymerases δ and ε.

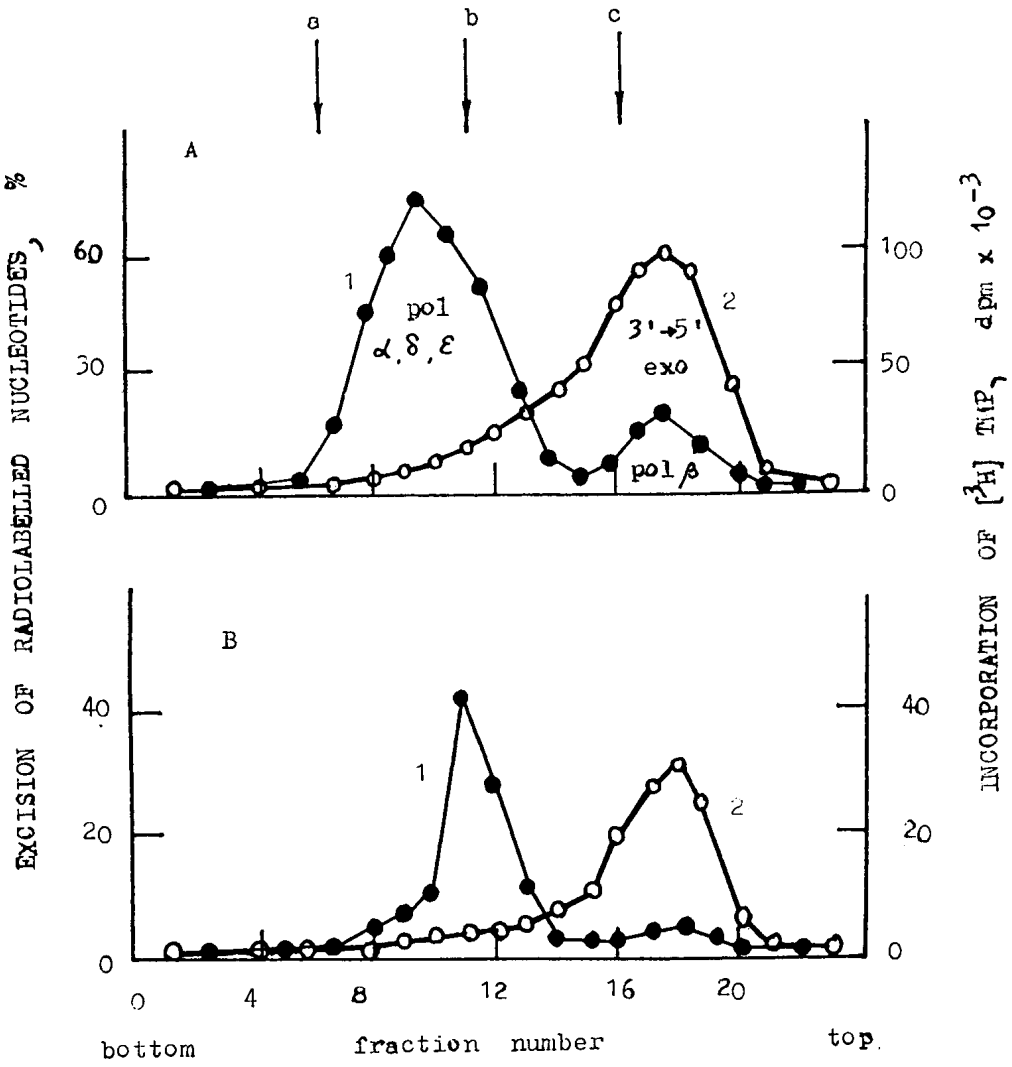

Fig. Sedimentation of rat cell-free extracts.
(A) nuclear extract from regenerating rat liver (similar results were obtained for normal liver, total embryos and for cytosol);
(B) Re-sedimentation of pooled fractions 12 and 13 from experiment (A), concentration of radiolabelled substrates and time of incubation were doubled as compared with experiment (A).
Curve 1, DNA polymerase activity;
Curve 2, $3' \rightarrow 5'$ exonuclease activity, 3'-end radiolabelled ssDNA being used as a substrate (excision from randomly labelled ssDNA has not exceed 5% throughout both sedimentogrammes).
a, catalase (240 kDa); b, aldolase (160 kDa); c, egg albumin (45 sDa)

So the autonomous nucleases, non-associated covalently with DNA polymerases, manifest a bulk of $3' \rightarrow 5'$ exonucleolytic activity in the extract from normal and regenerating rat liver as well as from total rat embryos. Hence, these exonucleases may be considered as the principal participants of "external proofreading".

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