CONTRIBUTION OF 3′ → 5′ EXONUKLEASES FROM RAT LIVER NUCLEI IN FIDELITY OF DNA SYNTHESIS CATALYZED BY MAMMALIAN DNA POLYMERASES α

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Mammalian nuclear DNA polymerases α and β are known to be devoid of editing 3′ → 5′ exonucleolytic activity, whereas this activity of DNA polymerases δ and ε is essentially lower than that of accrate prokaryotic DNA polymerases [1]. Exonucleases non-associated covalently with DNA polymerases have been suggested to proofread in mammalian nuclei. The proper exonucleases were found in the chromatin from rat liver [2,3]. Now we have isolated two 3′ → 5′ exonucleases with molecular masses of 40 and 50 *kD*a (exo-40 and exo-50) and purified them to near homogeneity [4]. They are shown to excise mismatched nucleotides from poly[d(A—T)] template respectively 10- and 2-fold faster than the matched ones [5—7]. Upon addition of any of these exonucleases to DNA polymerase α from rat liver or calf thymus, the fidelity of in vitro reproduction of primed DNA from bacteriophage φ X 174 amber 3 is increased 5—10-fold, the levels of exonuclease and polymerase and polymerase activities being approximately the same [8]. The ratio of exonuclease activity to polymerase one is lower for mutator and higher for antimutator strains of phage and bacterium [1]. Similar mutants have not been otained in the case of mammalian cells as yet. However, the levels of this ratio can be measured in mammalian subcellular preparations (free of endonuclease) to evaluate meaningfulness of potential proofreading into living mammalian cells [9]. The extrapolation of the correction

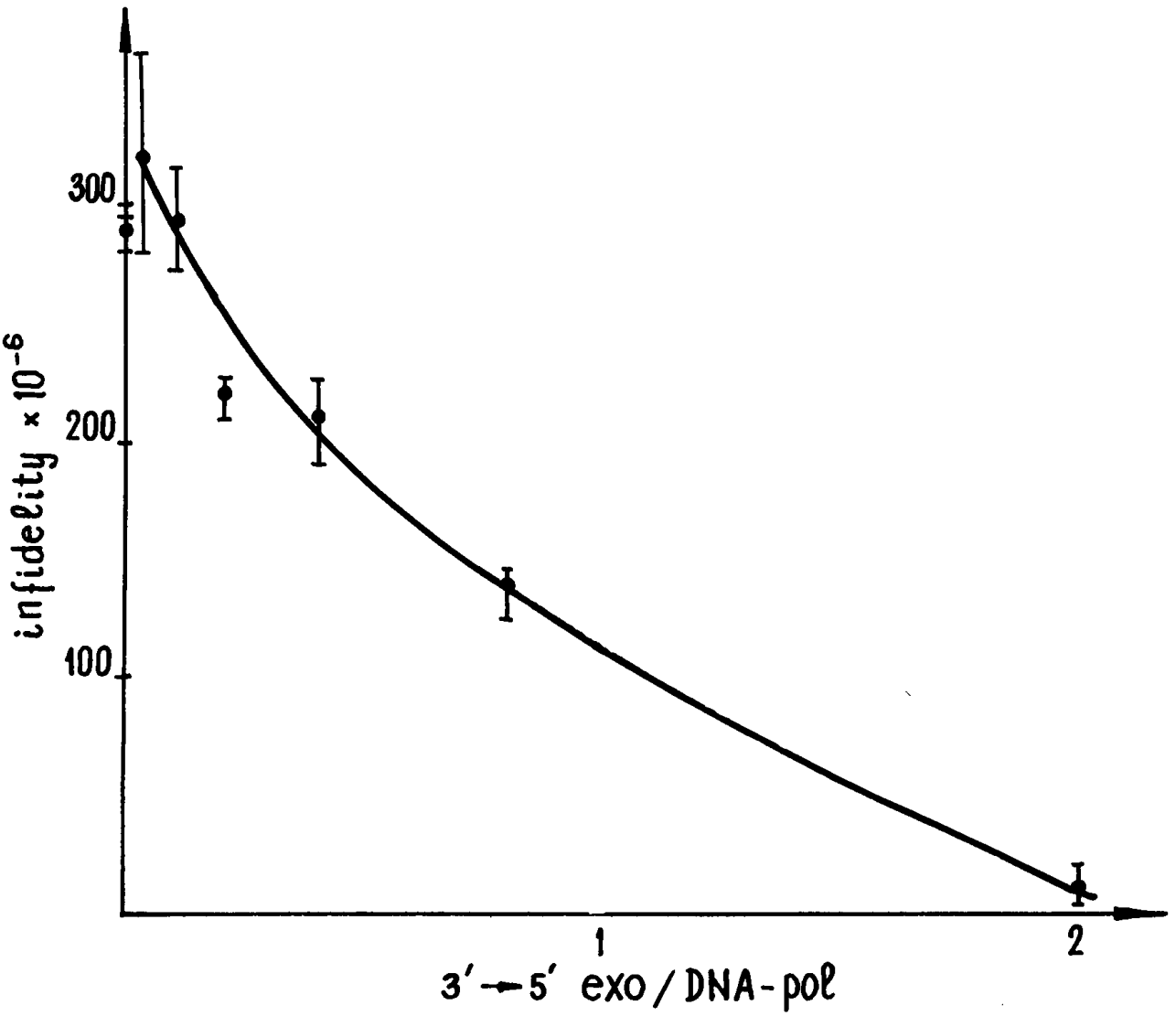

Fig. Dependence of infidelity of DNA syntesis (measured as the frequency of reversion of phage φ X 174 amber 3) on value of exo-50- activity to DNA polymerase activity.

Reversion has been counted as the ratio of phage titers on E. coli Sup^- to Su p^+ after DNA synthesis in vitro at "bias" of dGTP

to cellular conditions is carried out with the data presented in the fig. (here concentration of DNA polymerase α equals 20 u/ml. Which is near to the tissue concentration). The computer analysis has shown that the curve in the fig. is described by equation: $y = a + b \cdot e^{-1.5x}$ where y is an overall infidelity, a is spheroplast infidelity ($7 \cdot 10^{-6}$), b is the infidelity of DNA polymerase α, and x is the ratio of activities of exonuclease to DNA polymerase (exo/pol). Replication occurs in chromatin and nuclear membrane where minimal value of exo/pol equals five [9]. Hence, the contribution of $3' \rightarrow 5'$ exonuclease proofreading to the overall fidelity of α-polymerase DNA synthesis in hepatocyte may amount to three orders of magnitude. DNA polymerases α and β copurify with $3' \rightarrow 5'$ exonucleases in the course of their fractionation according to the size, charge, and hydrophobicity [8—10]. The copurification is not observed in the presence of 5% Triton X-100 (therefore, DNA polymerases δ and ε are absent) and is observed again after its removal [4,11]. Presumably $3' \rightarrow 5'$ exonucleases are constituents of replicative complexes [12].

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