# KINETICS OF REASSOCIATION OF DNA FROM THE THYMUS AND ASCITES HEPATOMA OF RATS

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The kinetics of renaturation of DNA from the rat thymus and ascites hepatoma was studied. Three zones were distinguished on the curve, corresponding to fast, intermediate, and slow rates of DNA reassociation. The course of the reassociation curves of DNA from tumor and normal tissues did not diverge in any of these zones. Consequently, no difference could be detected between the DNA's of normal and neoplastic cells on the basis of their reassociation kinetics.

KEY WORDS: normal cells; tumor cells; DNA; molecular hybrids.

Recent work has established differences in the ability of DNA from neoplastic and normal mammalian cells to form molecular hybrids with messenger RNA (mRNA) [5, 6]. It has also been shown that DNA's from certain strains of mouse myeloma differ in the number of sequences of immunoglobulins complementary to individual mRNAs [7]. However, it is not yet clear whether this phenomenon is connected with differences in the assortment of mRNA's synthesized in the cells or with changes in the structure of the DNA template.

In this investigation an attempt was made to determine the structure of the genome of rat thymus cells and ascites hepatoma cells from the same species of animals by determining the kinetics of renaturation of their DNA.

## EXPERIMENTAL METHOD

Noninbred male albino rats were used. The animals were inoculated intraperitoneally with cells of Zajdela's ascites hepatoma. On the 5th and 6th days after inoculation, thymidine-$H^3$ was injected. The total dose of thymidine was 500 $\mu$Ci per rat.

Nuclear DNA from the thymus of intact rats and the hepatoma cells was obtained as described previously [1]. Ultrasonic disintegration of the DNA was carried out on the UZDN-1 apparatus (15 kHz, 0.2 A) until fragments containing about 500 pairs of nucleotides were obtained. Solutions of the fragmented DNA were subjected to thermal denaturation. Renaturation of the DNA was carried out in 0.14 M phosphate buffer, pH 6.8, at 60°C in the following experimental variants: 1) unlabeled ascites hepatoma DNA, 2) a mixture of 90% thymus DNA and 10% hepatoma DNA labeled with thymidine-$H^3$. In the renaturation experiments DNA was used in concentrations of between 0.02 and 10 mg/ml. Analytical fractionation of single- or double-helical DNA was carried out in volume with the aid of hydroxyapatite (HA) [2, 3]. The HA was synthesized by the method described earlier [11]. The ratio between the renatured and native DNA's was calculated in per cent on the basis of the spectrophotometric data [2, 12]. In the case of labeled DNA, the radioactivity of the two fractions also was counted in a liquid scintillation counter (Nuclear Chicago, Mark II).

The kinetics of DNA renaturation was described in the system of coordinates suggested by Britten and Kohne [4].

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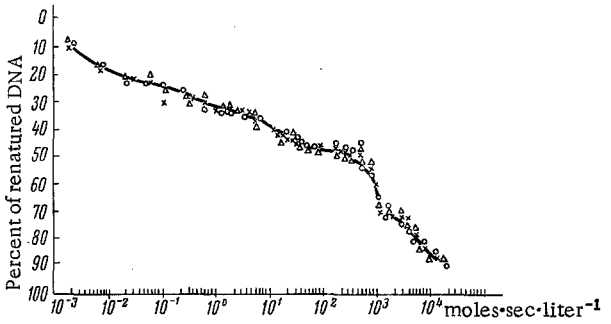


Fig. 1. Kinetics of renaturation of DNA from thymus and ascites hepatoma of rats. Crosses denote renaturation of DNA of ascites cells based on ultraviolet absorption; triangles denote renaturation of thymus RNA based on ultraviolet adsorption; circles denote renaturation of DNA of ascites cells based on level of radioactive label.

## EXPERIMENTAL RESULTS

The kinetics of renaturation of the rat ascites hepatoma DNA and the mixture of thymus DNA with labeled hepatoma DNA is illustrated in Fig. 1. The course of the renaturation curves shows the essential heterogeneity of rat DNA. Three principal zones corresponding to "fast," "intermediate," and "slow" rates of renaturation can be clearly distinguished. This agrees with the results of investigations carried out on DNA from various rat tissues [8, 10]. The "fast-renaturing" fraction accounted for 10-18%, the "intermediate" fraction for 20-25%, and the "slow" fraction for 55-60%. Closely similar values were obtained by Szala and Chorazy [10].

It is important to emphasize that the reassociation curves obtained for the thymus and rat tumor DNA's practically coincided in all three zones. Better agreement between the course of the curves also was observed when thymus DNA was reassociated when mixed with labeled hepatoma DNA. No divergence of the course of the curves could be detected with reference to the points obtained by calculating the ratios between native and denatured DNA on the basis of the spectrophotometric data and the measurements of radioactivity. These results indicate that no sequences are lost in the neoplastic DNA or that they are present in significant excess. Consequently, no difference between the DNA'S of normal and neoplastic cells can be detected on the basis of their renaturation kinetics. This, however, does not prove that there is no difference. Differences could exist as changes in individual sequences of base modification or as other defects not detectable by the method used. The possibility likewise cannot be ruled out that differences between neoplastic and normal cells are found only at the level of regulation of genome activity, as has been described in DNA : RNA hybridization experiments [6, 7]. Comparison of renaturation of DNA's from normal and neoplastic cells is also important from the aspect of the use of neoplastic DNA in hybridization experiments, for it is difficult to obtain effectively labeled DNA of slowly dividing cell populations. Mori et al. [9] recently showed that DNA from mouse liver and mouse plasma cytoma have a very similar renuturation kinetics. These observations, together with the results described in this paper, are evidence that highly labeled DNA from tumor cells can be used in molecular hybridization experiments with RNA obtained from tissues with low proliferative activity.

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