REASSOCIATION OF NORMAL MOUSE DNA AND MOUSE PLASMOCYTOMA DNA

K. MORI[1], M. WINTZERITH[2] and P. MANDEL[3]

Centre de Neurochimie du CNRS, Institut de Chimie Biologique, Faculté de Médecine, Université Louis Pasteur et Unité de Recherches Fondamentales sur la Biochimie de la Cellule Cancéreuse de l'INSERM, 11 rue Humann–67085 Strasbourg Cedex, France

Received 8 April 1973
Revised version received 10 July 1973

1. Introduction

Recently, studies of renaturation kinetics of DNA have been most useful in furthering our knowledge of different genomes [1–12]. In this note we present results of renaturation studies carried out on DNA from normal and tumoral mouse tissue. For many experiments it would be more convenient to use tumoral cell DNA than normal tissue DNA since tumour DNA displays the property of very high labelling possibilities compared to that of normal cells of the same animal. Thus it is possible to perform hybridization experiments using low quantities of labelled DNA with a very high ratio RNA/DNA in order to obtain a saturation state even when low quantities of RNA are available. It is obvious that prior to such investigations it is necessary to see if there are differences in these DNA's from the same animal. One of the criteria of these studies may be the reassociation kinetics.

2. Materials and methods

Balb/c strain mice were used in these experiments. Solid MPCII plasmocytoma cells were transplanted two weeks before the animals were killed. DNA was labelled *in vivo* by injection of [^{3}H]methyl-thymidine (300 μCi/mouse, CEA France) 38 hr before sacrifice. Livers and tumours were rapidly removed after decapitation of the mice. Nuclei were immediately isolated either from liver by the classical method of Chauveau et al. [13] or from tumours after homogenization of the tissue with an Ultraturrax apparatus in Tyrode solution (10 ml/tumour) in the presence of 0.1% Triton X-100 for 10 sec. Tumour nuclei were pelleted at 600 *g* 10 min at 0°C then repeatedly washed with fresh Tyrode buffer until the nuclei seemed pure by phase contrast microscopy.

The method used to prepare DNA was essentially that of Marmur [14] with minor modifications. After RNAase action, samples were treated with pronase (250 μg/ml) for 24 hr at 37°C in order to completely destroy the ribonuclease. Satellite-free DNA was prepared [15] by removing the satellite DNA (reassociated at Cot 10 by hydroxyapatite chromatography. The satellite-free DNA was purified on CsCl gradients.

DNA, dissolved in 0.12 M sodium phosphate buffer (pH 6.8) to an absorbance of 2–4 at 260 nm, was sheared as described previously [16] by sonication using an MSE sonicator. When reassociations were performed on cold liver DNA (99.4%) and labelled tumour DNA (0.6%), the DNA's were mixed prior to sonication.

The fragmented DNA samples were denatured in a boiling water bath for 10 min just before reassociation at 65°C in sodium phosphate buffer (pH 6.8) at the desired Cot value. When reassociations were carried out at an other ionic strength then that of standard buffer (0.12 M sodium phosphate buffer pH 6.8),

[1] Present address: Department of Neuropsychiatry, Osaka City University, Medical School, Osaka, Japan.
[2] Chargée de Recherche au CNRS - From whom reprints must be requested.
[3] With the technical assistance of M.C. Wermelinger and A. Staub.

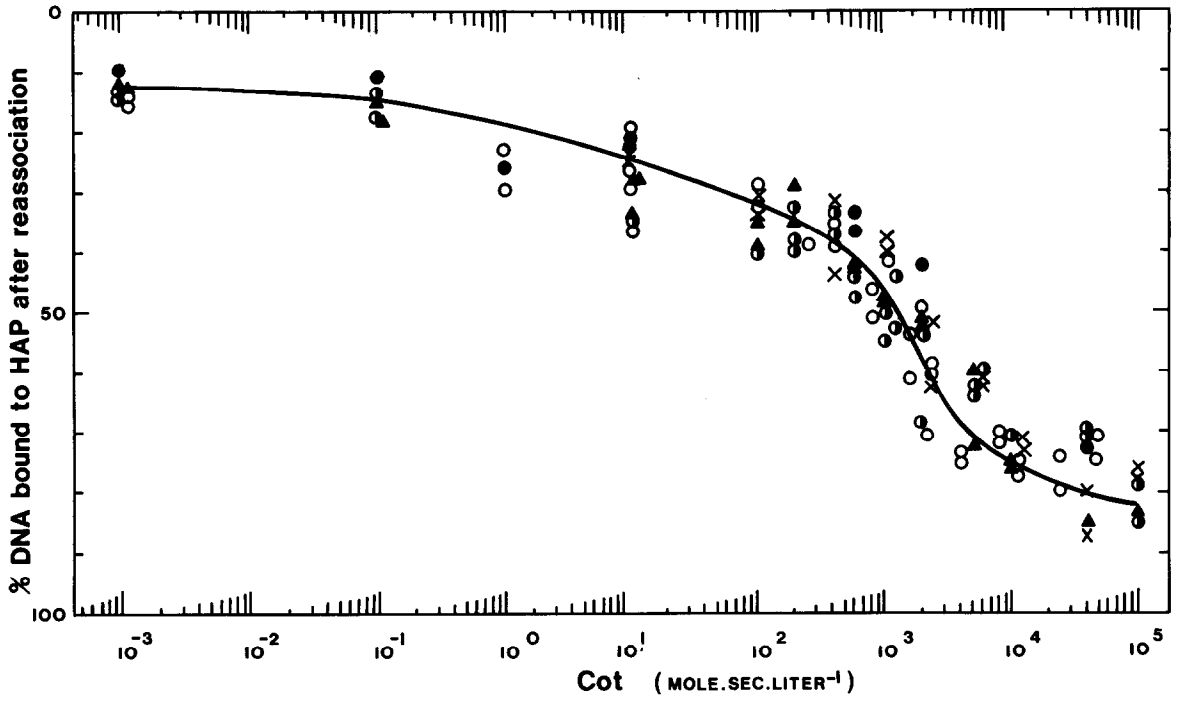

Fig. 1. Reassociation curves of mouse liver and tumour DNA. The abscissa scale is logarithmic and gives the Cots of the incubations. Following Britten and Kohne [11] the Cot is defined as the product of the DNA concentration (Co) and the time (t) of reassociation expressed as mole nucleotide x seconds x liter^{-1}. The ordinate scale is linear and gives the percent of reassociated DNA–DNA duplexes measured as the DNA-bound to hydroxyapatite: (—o—o—) Liver DNA–liver DNA; (—•—•—) tumour DNA–tumour DNA; (—x—x—) liver DNA without satellite–liver DNA without satellite; (—o—o—) liver DNA–tumour DNA. UV results; (—▲—▲—) liver DNA–tumour DNA. Radioactivity countings.

especially for high Cots, the correcting factors of Britten and Smith [17] were applied.

After incubation at 65°C, the DNA was fractionated on hydroxyapatite columns maintained at 60°C as previously described [16], into single stranded DNA, eluted with 0.12 M sodium phosphate buffer (pH 6.8), and DNA–DNA duplexes, eluted with 0.25 M sodium phosphate (pH 6.8). The percent of reassociated DNA–DNA duplexes was determined by UV measurements or by radioactivity. Optical densities were determined at 260 nm in a 2 cm length cell with a UV spectrophotometer Zeiss PMQII. Radioactivity was counted in an Intertechnique SL40 spectrometer using a 0.4 g % omnifluor (NEM) in toluene scintillator.

Tm's were determined by thermal elution with 0.12 M sodium phosphate buffer of the reassociated duplex bound on hydroxyapatite column and measured by radioactivity in Instagel (Packard) scintillator. To insure total recovery a final elution with 0.25 M phosphate buffer was carried out. The temperature in °C at which 50% of the duplex elutes corresponds to the Tm.

3. Results and discussion

The reassociation curves (fig. 1) are those commonly obtained for mouse DNA (EC 4.6.11.12). The reassociations between liver DNA–liver DNA, tumour DNA–tumour DNA, liver DNA without satellite DNA, and liver DNA–tumour DNA, were very similar in the Cot range 400–11 000 where the sequences which are not highly repetitive reassociate. The slopes of the regression lines were determined (Programma 201 Olivetti). The Student–Fisher test applied to these figures did not reveal significant differences ($p > 0.05$).

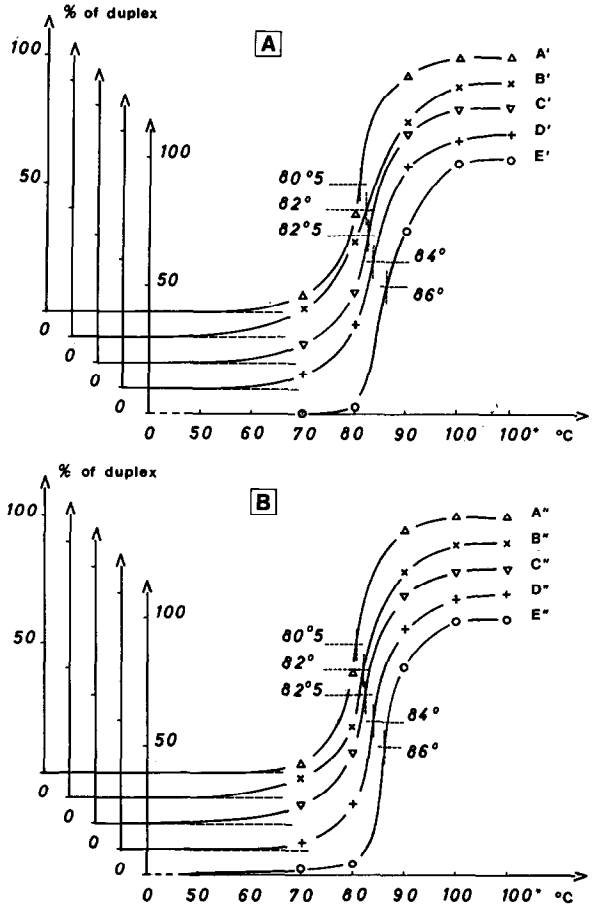

Fig. 2. Tm's of reassociated DNA–DNA duplexes: A) from tumour; B) from liver, at different Cots. A', A'': Cot 10; B', B'': Cot 3000; C', C'': Cot 900; D'D'': Cot 18000; E', E'': native sonicated DNA. Tm's were determined by thermal elution from hydroxyapatite columns as described under Materials and methods. At temperature 100*°C a final elution with 0.25 M sodium phosphate buffer was carried out instead of 0.12 M sodium phosphate buffer as for the other temperatures.

Thus it appears that tumour DNA reassociated like liver DNA when the two homologous strands of the whole nuclear liver DNA, or satellite deprived liver DNA were used for reassociation. Moreover when 'heterologous' reassociation was performed (labelled tumour DNA and an excess cold liver DNA) the fraction of labelled duplexes which reassociated (essentially heteroduplexes liver DNA–tumour DNA) is similar to that obtained simultaneously with liver DNA, the measurements being done by UV. These data suggest, as shown for L cells by Britten and Rake [2], neither loss of sequences, nor appreciable presence of supernumerary sequences, in tumour DNA as compared to normal DNA.

Tm's of the reassociated duplexes at different Cots were studied. The results were shown in fig. 2. Tm's were lower than that of native DNA by 2–5°C and increased with the incubation Cot in tumoral and normal DNA duplex. Moreover the same Tm's were found at a given Cot for tumour DNA duplexes as for liver DNA duplexes. Our data did not show quantitative (Cot curve) nor qualitative (Tm) differences for normal and tumoral DNA in terms of DNA reassociation. This might have been observed since the tumours used contain an RNA virus, and a DNA sequence produced by enzymic RNA-directed DNA synthesis could be incorporated into the tumour DNA. Since the reassociations were similar for DNA of normal and tumoral tissue of the same strain, plasmocytoma tumour can be used as a good starting material for reassociation and hybridization studies on mouse genome

Acknowledgements

The authors are especially indebted to Dr. I.G. Morgan for his help with the English in this manuscript.

References

[1] Britten, R.J. and Kohne, D.E. (1968) Science 161, 529.
[2] Britten, R.J. and Rake, A.V. (1968) Carnegie Inst. Wash. Yearbook 67, 325.
[3] Wetmur, J.G. and Davidson, E.H. (1968) J. Mol. Biol. 31, 346.
[4] Gelderman, A.H., Rake, A.V. and Britten, R.J. (1968) Carnegie Inst. Wash. Yearbook 67, 320.
[5] Davidson, E.H. and Hough, B.R. (1969) Proc. Natl. Acad. Sci. U.S. 63, 342.
[6] Laird, C.D. (1971) Chromosoma 32, 378.
[7] Lambert, C.C. and Laird, C.D. (1971) Biochim. Biophys. Acta 240, 39.
[8] Straus, N.A. (1971) Proc. Natl. Acad. Sci. U.S. 68, 799.
[9] Gelderman, A.H., Rake, A.V. and Britten, R.J. (1971) Proc. Natl. Acad. Sci. U.S. 68, 172.
[10] Khoury, G. and Martin, M.A. (1972) Nature New Biol. 238, 14.
[11] Hahn, W.E. and Laird, C.D. (1971) Science 173, 158.

[12] Grouse, L., Chilton, M.D. and McCarthy, B.J. (1972) Biochemistry 11, 798.
[13] Chauveau, J., Moulé, Y. and Rouiller, C. (1956) Exptl. Cell Res. 11, 317.
[14] Marmur, J. (1961) J. Mol. Biol. 3, 208.
[15] Wintzerith, M., Mori, K. and Mandel, P., submitted to J. Neurochem.
[16] Mori, K., Wintzerith, M. and Mandel, P. (1972) Biochimie, 54, 1427.
[17] Britten, R.J. and Smith, J. (1969) Carnegie Inst. Wash. Yearbook 68, 378.