COMPLEXES OF POLYOMA DNA AND SYNTHETIC POLYRIBONUCLEOTIDES

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Summary: The complexes formed between polyoma DNA and polyribonucleotides indicate that this DNA contains both cytosine-rich and thymine-rich clusters. Their even distribution precludes strand separation by this method. The pyrimidine-rich clusters cannot be easily correlated with the promotion of the polycistronic transcription of polyoma DNA.

Complex formation of DNA with guanine- or uracil-containing polyribonucleotides seems to be an indication of the distribution, in the two strands, of cytosine- or adenine-rich clusters, respectively[1]. In bacteria and phages, cytosine-rich clusters are thought to be "promoters" of transcription[1], the strand containing them being the one transcribed in vivo[2]. A similar analysis of pyrimidine cluster distribution in a small animal virus DNA is described here.

To separate polyoma DNA I from DNA III (mouse DNA of pseudovirions[3]), 1 ml (up to 5×10^6 hemagglutination units) of purified[4] virus in 0.2 M NaOH[5] was layered over 12 ml of an alkaline CsCl gradient and centrifuged for 3 hours at 37,000 rpm in a Spinco SW40 rotor. The DNA I (53 S) was treated with hydroquinone[6] or alkali[2]; the resulting mixture of linear 16 S and circular 18 S DNA was isolated by the same sedimentation procedure. Phage $\lambda cb_2^+ \cdot K$ (a plate lysate was kindly provided by Prof. W.Arber) was purified by equilibrium banding in CsCl. Poly G (Miles) was partially degraded[7] and chromatographed on DEAE-cellulose in 7 M urea. The poly G fraction eluting between 0.38 and 0.52 M NaCl sedimented with 3.7 S (uncorrected, at 23°C in CsCl of 1.353 g/ml density). Poly (I,G) (1:1), a kind gift of Dr P.Sheldrick, was hydrolyzed for 0-32 min. at 25°C in 0.1 M NaOH; 2 samples (10 and 20 min.) sedimented with about 4 S. I thank Dr H.Kubinski for samples of poly (U,G) (1:1) and poly U, already checked for strand separating efficiency[2]. In order to check the poly G, control experiments

were done with λ DNA. The ribopolymer and $2x10^{12}$ intact phage in 40 µl were incubated with 0.12 ml 5 mM Na_2EDTA, 0.1 % Sarkosyl NL97 (Geigy), pH 9 for 3 min. in a boiling water bath and quenched in ice water. Complexes of polyoma DNA were formed by similarly heating 2-4 µg DNA and the ribopolymer in about 0.14 ml with 20 µl 50 mM Tris-Cl, 10 mM Na_2EDTA, pH 7.4[8]. The ribopolymers were used in the following proportions, with respect to DNA: poly (I,G), 10-, 30-, or 50-fold; poly U, 10- or 50-fold; poly (U,G), 5- or 10-fold; poly G, equimolar, 2-, or 3-fold. The same density shifts of DNA were observed for all ratios used, indicating that the complexing sites on the DNA were saturated with the ribopolymer[7]. After the addition of CsCl, the refractive index was checked, and the sample centrifuged for at least 16 hours at 44,770 rpm and 20°C in a Spinco An-D rotor. Multiple tracings of the ultraviolet photographs were superimposed. Densities were calculated with the aid of experimental gradient correction factors[9]. No density markers were used; the standard errors of 1-2 mg/ml, found for the density values given in the text (averaged from different experiments such as those shown in the figures) do not affect the results.

Both λ and polyoma DNA, heat denatured in the absence of ribopolymers, banded at 1.710 g/ml (Fig.1a,b). The experiment described in Fig.1c shows that the heating procedure denatured the DNA completely. In accordance with previous findings[2], most of the complex of λ DNA with poly G formed two equal peaks with densities of 1.716 and 1.724 g/ml (Fig.2a), while 5-10 %, presumably renatured DNA, was found at 1.698 g/ml.

Most of the polyoma DNA-poly G complex was found in a single band at 1.726 g/ml (Fig.2b). A slight skewing on the heavy side indicated the presence of 5-10 % of a heavier material. Variable amounts were occasionally found at a lighter density (up to 20 %, Fig.2b, inset), corresponding to that of denatured DNA. With poly (I,G), polyoma DNA produced a main band at 1.726 g/ml and a shoulder (10-20 %) at 1.741 g/ml (Fig.2c). This pattern is similar to the one obtained with poly G. Complexes of polyoma DNA with poly U or poly (U,G) showed broad bands at 1.734 and 1.753 g/ml, respectively (Fig.3), but no incipient resolution into distinct peaks was evident. The density increase due to poly (U,G) was about the

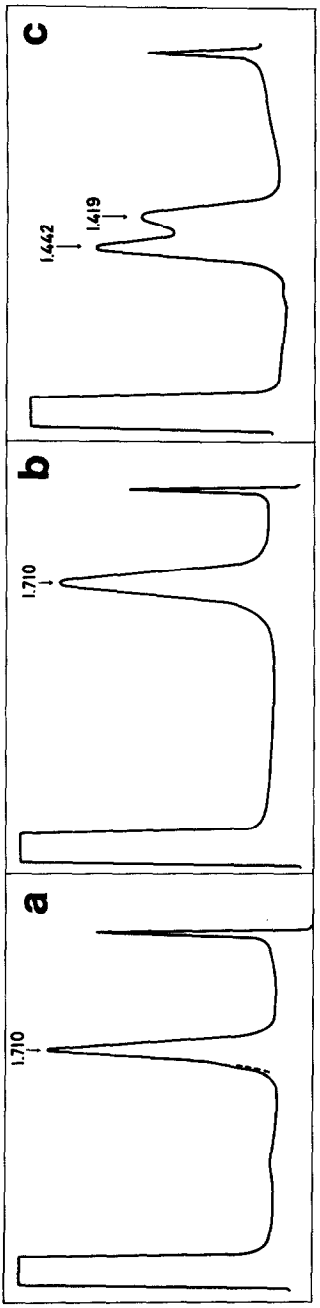
c
1.442
1.419
b
1.710
a
1.710

Fig. 1

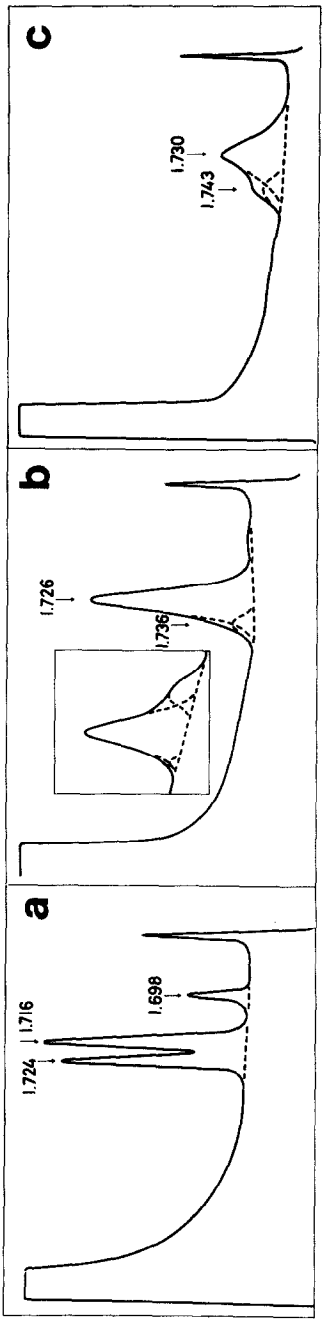
c
1.730
1.743
b
1.726
1.736
a
1.724
1.716
1.698

Fig. 2

Fig.1: Analytical centrifugation patterns (numbers denote densities, with respect to the value of 1.7035 g/ml for Escherichia coli DNA[9]): (a) 1.5 μg heat-denatured λ DNA, in CsCl. (b) 2.4 μg heat-denatured polyoma DNA, in CsCl. (In CsCl, both λ and polyoma DNA were found to be 7-8 mg/ml lighter than expected[9-12], while renatured λ DNA (Fig.2a) was 5 mg/ml lighter than expected for native DNA[9]). (c) 2.6 μg partially renatured polyoma DNA, in Cs_2SO_4 (which provides a better separation between native and denatured DNA than CsCl); after denaturation, only one band at 1.440 g/ml was found.

Fig.2: Analytical centrifugation patterns of heat-denatured and quenched mixtures of DNA and ribopolymers, in CsCl (numbers denote densities): (a) 1.5 μg λ DNA + 2.8 μg poly G. (b) 1.4 μg (inset 1.8 μg) polyoma DNA + 2.6 μg poly G. (c) 1.3 μg polyoma DNA + 13 μg poly (I,G) (32 min. OH^-).

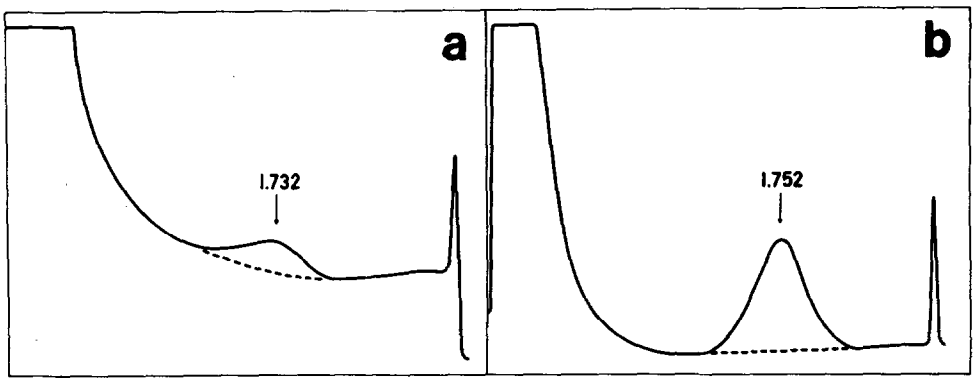

Fig.3: Analytical centrifugation patterns of heat-denatured and quenched mixtures of DNA and ribopolymers, in CsCl (numbers denote densities): (a) 0.75 μg polyoma DNA + 36 μg poly U. (b) 2.4 μg polyoma DNA + 24 μg poly (U,G).

sum of those due to poly U and poly G. Since a significant broadening of a DNA peak is associated with the complex formation itself[13], a further investigation of the broad bands was not undertaken. Since no patterns comprising two peaks of equal size were produced, it seems that the two strands of polyoma DNA cannot be separated by complexing with any homopolyribonucleotides or copolymers of random sequence. In some preliminary experiments, no strand separation was achieved, either, by binding mercuric or silver ions to denatured polyoma DNA in borate; but a separation should be possible by hybridization with the polycistronic[14] polyoma messenger RNA.

The following discussion of the occurrence of pyrimidine-rich sequences is, of course, limited to the rather large cytosine-rich and/or thymine-rich clusters detectable by the complexing method used[1]. No conclusions can be drawn regarding the presence in the DNA of any short, specific nucleotide sequences, which might not be detectable by this method, but which might be of importance for transcription. The single-band complexes formed indicate that polyoma DNA does not contain the highly asymmetric distribution of pyrimidine clusters characteristic of many bacterial and phage DNA's[1], including the replicative form DNA's of the small phages M13 and ØX174[13]. The density increment for polyoma DNA, complexed with poly (I,G) or poly G, corresponds to that of the dense strand of λ DNA. Thus, if one assumes that cytosine-rich clusters are of comparable sizes in the two DNA's, their average number per unit length will also be the same. According to estimates for this number, made for various DNA's[1,13], polyoma DNA would contain either 2 or 4 cytosine-rich clusters, symmetrically distributed between the two strands. The complexing with poly U indicates[1] that adenine-rich (and therefore thymine-rich) sequences are present in polyoma DNA, and that their number, again, is about the same in both strands.

Considering the results obtained with λ DNA[2], the appeal of Szybalski's hypothesis[1] regarding a promoter function of cytosine-rich clusters is obvious. But since a major part of the polyoma genome seems to be transcribed in one piece[14], one has to conclude that pyrimidine-rich clusters cannot promote polyoma transcription - unless the transcription of a very short piece of one strand and that of the major part of the other strand are dependent on one cluster each. In the case of the related SV40 DNA, cytosine-rich clusters, which have been implicated in bacterial and phage transcription, have not been detected, as judged by complexing with poly (I,G)[15], while similar amounts of thymine-rich clusters were found in both strands, using poly U[15,1]. If Szybalski's hypothesis is correct, different control mechanisms would probably have to be postulated for transcription, one operative in phages and bacteria, and the other in polyoma and related viruses and perhaps their hosts. (In mitochondrial DNA from rat liver, thymine-rich, but not cytosine-

rich, clusters might conceivably control transcription[16]). From base doublet frequency patterns, Subak-Sharpe[17] concluded that it is highly probable that polyoma and related viruses originated from animal cells and the phages from bacteria. The two groups might thus also have different promotion mechanisms for transcription.

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