DEOXYADENYLATE-RICH SEQUENCES IN MAMMALIAN DNA

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1. Introduction

Adenylate-rich sequences have been observed in heterogeneous nuclear RNA [1–4], in the rapidly labelled polyribosomal RNA and messenger RNA of mammalian cells [1–9] and also in virus-specific messenger RNA [10, 11]. It is still not clear whether such sequences arise directly as a result of transcription from DNA [1, 3, 10] or are added during a post-transcriptional process [2,4,11]. One problem is whether deoxythymidylate-rich (dT-rich) regions exist in mammalian DNA, which might serve as a template for the adenylate-rich (rA-rich) sequences in RNA. Possible detection of dT-rich sequences through the formation of hybrids with polyadenylic acid (poly rA) is complicated by the resistance of poly rA to pancreatic ribonuclease, and the fact that poly rA itself binds to nitrocellulose filters [7]. The complementary deoxyadenylate-rich (dA-rich) sequences can however be detected in mammalian DNA by hybridisation with ^{3}H-labelled polyuridylic acid (^{3}H-poly U) and the characteristics of this hybridisation reaction are now reported.

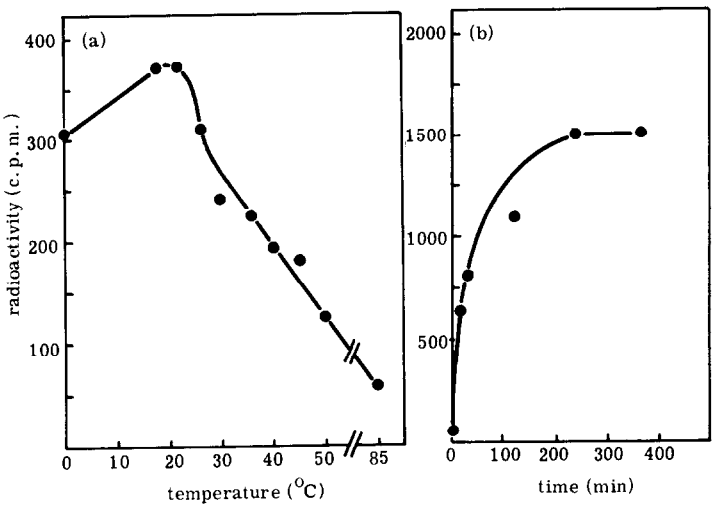

Fig. 1. Effect of temperature and time of incubation on BHK-21/C13 DNA ^{3}H-poly U hybrid formation. Each incubation mixture contained 5 μg denatured BHK-21/C13 DNA and 0.07 μg ^{3}H-poly U in 0.55 ml 2 × SSC. In (a) mixtures were incubated for 20 min at different temperatures, whereas in (b) mixtures were incubated at 20° for different periods. After these treatments, the mixtures were quickly cooled, 5 ml of ice-cold 2 × SSC were added and the hybrids were collected and estimated as described in Materials and methods.

2. Materials and methods

DNA was prepared from baby hamster kidney cells (BHK-21/C13), Krebs II mouse ascites tumour cells, mouse spleen, *E. coli*, *M. lysodeikticus*, and *Cl. perfringens* by the method of Marmur [12]. Calf thymus and salmon sperm DNA were purchased from Worthington Biochemicals Corporation. The relaxed circular form of SV40 DNA was kindly donated by Dr. R. Eason, and ØX174-replicative form relaxed circular DNA, phage λ DNA and phage T7 DNA were gifts from Dr. A.M. Campbell and Mr. D. Jolly, all of this Department. ^{3}H-poly U (M.W. > 50,000; specific activity 600,000 dpm per μg) was obtained from Miles Laboratories.

When hybridisation was performed in the liquid phase, DNA was denatured by heating to 100° in 0.1 × SSC (hyperchromicity 25–40%), hybridisation performed in 2 × SSC (1 × SSC is 0.15 M sodium

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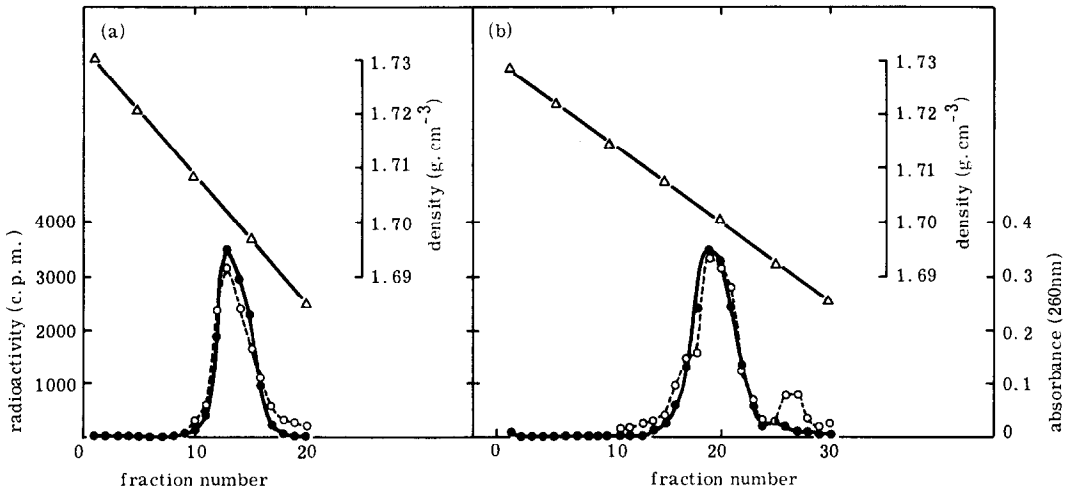

Fig. 2. Hybridisation of ^{3}H-poly U to CsCl gradient fractions of DNA. Following the general technique of Birnstiel et al. [14], 100 μg of DNA was centrifuged to equilibrium in CsCl at 33,000 rpm for 64 hr in a Spinco no. 40 angle-rotor. DNA fractions were collected into 0.1 × SSC, their absorbance determined at 260 nm, denatured in alkali, and then bound to Millipore filters (10 mm). The DNA-loaded filters from each gradient ((a) BHK-21/C13 DNA (b) Krebs II cell DNA) were hybridised against 1.5 μg ^{3}H-poly U in 1.5 ml 2 × SSC for 4 hr at 20°, and then washed extensively and treated with pancreatic ribonuclease (5 μg/ml) as in Materials and methods. Absorbance at 260 nm (•—•—•); ^{3}H-radioactivity due to hybrids (o---o---o); density of CsCl (Δ–Δ–Δ).

chloride–0.015 M trisodium citrate pH 7.0), and hybrids collected on nitrocellulose membranes [13], non-hybridised ^{3}H-poly U being removed by treatment with boiled pancreatic ribonuclease (5 μg/ml at room temp for 30 min). When hybridisation was carried out on nitrocellulose filters, the method of Birnstiel et al. [14] was used for binding of alkali-denatured DNA, and for processing of filters. After drying, the filters were assayed for radioactivity in toluene based scintillator [15].

3. Results

^{3}H-poly U hybridises very readily to denatured BHK-21/C13 DNA. The optimal temperature for hybridisation was found to be 18–22° (fig. 1a) and 20° was chosen as the standard temperature for all subsequent hybridisations. The time course of hybridisation with a ^{3}H-poly U: DNA ratio of 0.07:5 (fig. 1b) demonstrates the rapidity of the reaction, the $C_0t_{1/2}$ [20] for poly U being 5.9×10^{-4} mole·sec·l^{-1}.

That the hybridisation observed was specifically to DNA rather than to contaminating sequences of poly rA was demonstrated in two ways. First, incubation of DNA in 0.5 N NaOH at 37° for 18 hr led to a fall of less than 5% of the hybridisation with ^{3}H-poly U. Secondly, when DNA was centrifuged to equilibrium in caesium chloride, all fractions across the DNA band, when denatured, showed an equal affinity for poly U (fig. 2).

The melting profile of the poly U·DNA hybrid was found to be broad and symmetrical with a T_m of 46° (fig. 3) and typical saturation experiments (fig. 4) showed saturation values of 4.2 ng poly U hybridised per μg BHK-21/C13 DNA, and 5.5 ng poly U hybridised per μg Krebs II cell DNA.

Subsequently, DNA from several sources was tested for the ability to hybridise poly U, the relative extent of hybridisation being summarised in table 1.

4. Discussion

Some years ago poly U was shown to complex to certain denatured DNAs and thus change their buoyant density [18], but the reaction involved was not fully characterised. The specific hybridisation of poly

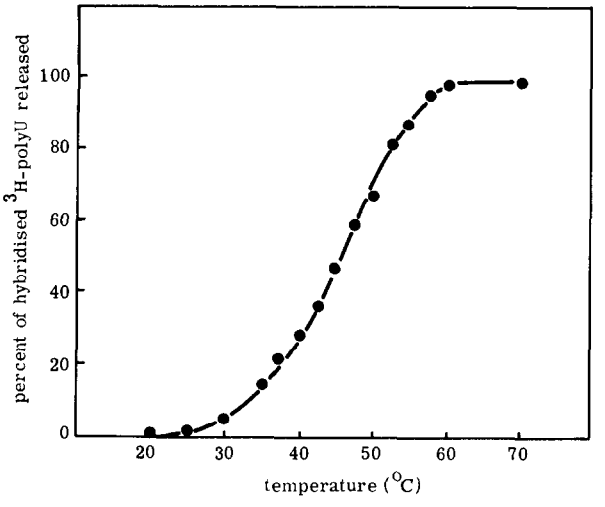

Fig. 3. The melting profile of the BHK-21/C13 DNA·^{3}H-poly U hybrid. 20 μg BHK-21/C13 DNA was bound to a 10 mm Millipore filter and incubated with 0.1 μg ^{3}H-poly U in 1 ml 2 × SSC for 4 hr at 20°. The acid-insoluble radioactivity released from the hybrid into 1 × SSC at increasing temperature was determined following the technique of Birnstiel et al. [17].

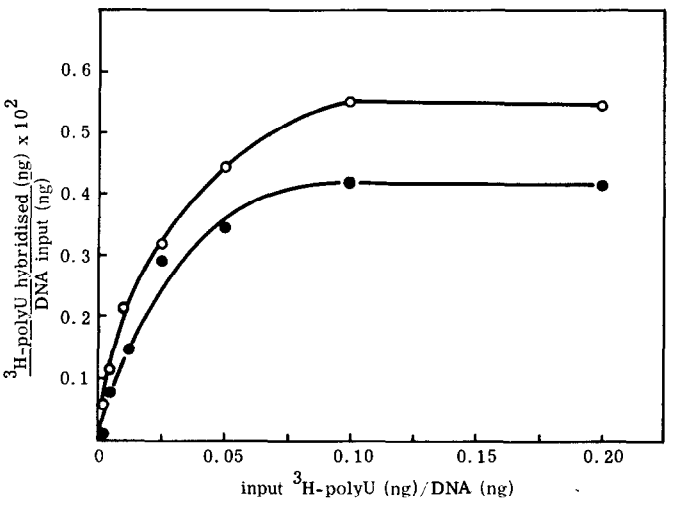

Fig. 4. 1 μg of heat denatured DNA was incubated with increasing amounts of ^{3}H-poly U in 1 ml 2 × SSC for 4 hr at 20°. Hybrids were estimated as in Materials and methods. Correction has been made for radioactivity bound to filters from control incubations with no DNA (0–25 cpm for different ^{3}H-poly U inputs). (○–○–○): hybridisation with Krebs II cell DNA; (●–●–●): hybridisation with BHK-21/C13 cell DNA.

U to denatured DNA of several species has now been demonstrated. For BHK-21/C13 and Krebs II DNA at least, density gradient analysis has clearly shown that polydeoxynucleotide sequences which hybridise to poly U are homogeneously distributed throughout DNA molecules, whatever their adenine plus thymine content. The minor peak of hybridisation found with Krebs II DNA at density 1.69 g·cm^{-3} may represent hybridisation to satellite DNA [19].

The broad melting profile of the hybrids would suggest some heterogeneity in the size of the poly U sequence hybridised [10]. The T_m of 46° is lower than that observed for synthetic hybrids between polydeoxyadenylic acid (poly dA) and poly U (about 53° [21]). This may be due either to a small average size of hybridised poly U molecules, or to about 10% mismatched base pairs in the hybrid [20]. In this context it should be noted that the rA-rich sequences in heterogeneous nuclear RNA and messenger RNA may contain up to 15% of other nucleotides [4, 5].

If poly U hybridises to poly dA regions in a 1:1 ratio, the fraction of DNA containing dA-rich sequences is 0.55% for Krebs II DNA and 0.42% for BHK-21/C13 DNA. Thus the total level of dA·dT-rich sequences in these DNAs would be approx. 1.1% for Krebs II DNA and 0.84% for BHK-21/C13 DNA. On the other hand it is possible that these estimates are high since hybrids between poly dA and poly U may well be formed in a ratio of 1:2 [21].

Finally, there is an apparent distinction between the extent of poly U hybridisation to DNA of mammalian origin on the one hand, and to DNA of bacteria or bacteriophage on the other. The significance of this is not yet obvious, but it might well be related to the occurrence of adenylate-rich sequences in mammalian RNAs, no such sequences having been reported as yet in bacterial RNA.

Acknowledgements

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Table 1
The relative hybridisation of ^{3}H-poly U with various DNAs.

DNA	^{3}H-radioactivity in hybrid (cpm)
BHK-21/C13	518
Krebs II mouse tumour	655
Mouse spleen	571
Calf thymus	299
Salmon Testis	45
E. coli	2
M. lysodeikticus	1
Cl. perfringens	47
SV40	307
ØX174 (replicative form)	5
Phage λ	84
Phage T7	0

1 μg of denatured DNA was incubated with 0.05 μg ^{3}H-poly U in 1 ml 2 × SSC for 4 hr at 20°. Hybrids formed were estimated as in Materials and methods. All experiments were performed in duplicate on two occasions and average results are shown. Correction has been made for radioactivity bound under control conditions with no DNA (average = 8 cpm).

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