PARTIAL HOMOLOGY BETWEEN RNA FROM RAUSCHER MOUSE LEUKEMIA VIRUS AND CELLULAR DNA

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Previous hybridization experiments have shown the existence of a partial homology between high molecular weight (hmw) RNA from Avian Myeloblastosis virus (AMV) or Rous Sarcoma Virus (RSV) and DNA from either normal or transformed cells (Harel et al., 1966 a, b). Since hmw RNA has been isolated from Rauscher Mouse Leukemia Virus (MLV) (Galibert et al., 1965, Duesberg and Robinson, 1966) it was of interest to perform similar experiments with this murine virus.

I - MATERIAL and METHODS : Virus producing JLS V5 cells (Wright and Lasfargues, 1965) were grown in Roux's flasks containing 60 ml Eagle's medium plus 10 % Calf serum. Nearly confluent monolayers were labeled by incubating in phosphate free Eagle's medium with 50 µc/ml of carrier free $^{32}PO^{4}H^{3}$, for 24 hours at 37°C. Virus was purified from the medium and viral RNA was extracted and fractionnated by gradient centrifugation as previously described (Huppert et al., 1966) Bulk cellular RNA and 4 - 5S RNA were prepared from FLS ascites tumor cells of Mouse (Harel et al., 1963). DNA was purified by the procedure of Marmur (1961), in the case of liver a preliminary extraction with phenol and 1 % Sodium dodecylsulfate was performed. Subsequent treatment of DNA, and annealing experiments using nitro-cellulose membranes were performed according to the method of Gillepsie and Spiegelman (1965), in liquid as previously described (Harel et al., 1966, b), including incubation with pancreatic ribonuclease (RNase). T_1 RNase was also used (Takadiastase sankyo 10 units/ml 40 minutes at 37°C). In some experiments annealed RNA was eluted, after incubation with pronase (Harel et al., 1966 a, b). Base compositions were determined by adding carrier RNA and applying previously described methods (Harel et al., 1963). In one experiment,

hybridized RNA was eluted, incubated with 200 µg carrier 4 - 5S RNA, 2 µg RNase free Deoxyribonuclease (Worthington) in Mg Cl^2 0.002 M, for 20 minutes at 37°C, reextracted with phenol, precipitated with ethanol, dissolved in 0.5 ml phosphate buffer 0.01M, EDTA 0.01M and centrifuged as indicated in the legend of the figure. Each fraction was measured at 260 mµ in a Zeiss spectrophotometer thereafter dried on a planchette (pretested for the absence of contaminant radioactivity) and counted twice for 60 minutes in a low back ground (0.3 - 0.4 cpm) Tracerlab flow counter.

II - RESULTS : In all experiments a large proportion of MLV RNA sedimented as a labeled peak of 70 - 75S. Only fractions greater than 60S were considered as hmw viral RNA, and utilized for hybridization after dialysis. These had a total radioactivity varying between 45,000 and 54,000 cpm.

Table 1 shows that in 7 experiments an average of 3.4 ± 0.5 % of input viral RNA was bound to Mouse DNA, and only 0.32 ± 0.05 % to E. Coli DNA. The latter figure was not much greater than that of other controls (no DNA, no incubation). RNase resistance of the annealed material was ascertained by repeating incubation with the enzyme or increasing the enzyme concentration. Hybridization yields appeared to be higher with Mouse DNA than with Chicken DNA, but such few comparative results may not be conclusive.

Experiment Nr	PER CENT INPUT VIRAL RNA HYBRIDIZED with 100 µg DNA from :		
	Mouse	Chicken	E. Coli
1 - 7 (means)	3.4 + 0.5	-	0.32 + 0.05
8	2.5	1.4 (a)	0.2
9	2.4	1.2 (a)	0.2
		2.1 (b)	

Table 1. - Hybridization yields of MLV RNA. Radioactive RNA inputs : 3,600 to 22,000 cpm (specific radioactivity greater than 30,000 cpm per µg). No significant differences were found using DNA from Mouse FLS ascites tumor or DNA from Mouse normal liver. a : DNA from Chicken leukemic cells, b : DNA from Chicken normal liver.

About 90 % of the radioactivity (600 to 1100 cpm), eluted from several membranes was acid precipitable, and was found in the 2' 3' ribonucleotides. Table 2 shows the base composition of hmw MLV RNA (which is in agreement with the data of Duesberg and Robinson) and that of its "core" as compared with the base composition of annealed viral RNA. The latter appeared strikingly different by its very high content of adelylic acid, and did not change much whether hybridized RNA had been treated with pancreatic or T_1 RNase.

	MOLES PER CENT				
	C	A	G	U	$\frac{A+U}{C+G}$
Viral RNA > 60S	25.4	25.5	26.5	22.5	0.92
Viral RNA "Core"	11.4	39.3	38.5	10.7	1.00
Annealed a	6.5	66.3	17.0	10.2	3.25
Viral RNA b	11.5	62.5	15.9	10.1	2.65

Table 2. - Base composition (means of two experiments). C : Cytidylic Acid, A : Adenylic Acid, G : Guanylic Acid, U : Uridylic Acid. "Core" : Acid precipitable fraction representing 24 % of the initial radioactivity of non annealed MLV RNA incubated with pancreatic RNase in conditions similar to those applied to annealed RNA a. Annealed RNA b was incubated with T_1 RNase.

		PER CENT INPUT RNA HYBRIDIZED in the presence of non labeled :		
Exp. Nr	Input viral RNA (cpm)	None	Cellular RNA (+ 10 µg, ++ 25 µg)	Poly A (10 µg)
1	3,600	2.5	+ 2.0	-
2	22,000	2.8	++ 2.5	-
3	24,00	2.5	+ 2.2	0.9
4	4,100	3.2	-	1.6

Table 3. - Competition experiments. Annealing with DNA from FLS Mouse tumor in exp. 1 - 3, DNA from Mouse liver in exp. 2 - 4. Incubation with pancreatic RNase in exp. 1 ₮ 2 - 3, T_1 RNase in exp. 4.

Table 3 gives the results of competition experiments. In relatively high concentrations cellular RNA did not markedly decrease the hybridization yields, whereas poly A exibited some competitive effect.

The figure shows the centrifugation pattern of annealed viral RNA which sedimented as a single peak ahead of 4 - 5S carrier RNA.

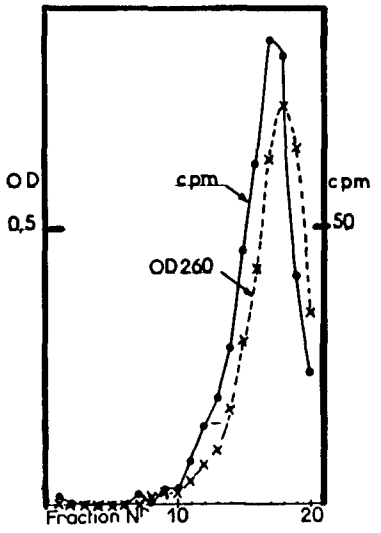

Figure. Hybridized fraction of viral RNA centrifuged in 5 - 20 % glycerol gradient, phosphate buffer 0.01M, EDTA 0.01M, for 90 minutes at 64,000 rpm in the SW 65 rotor of Spinco ultracentrifuge. Solid line : radioactivity of viral RNA. Broken line : A 260 of carrier RNA.

III - DISCUSSION : The present results demonstrate some homology between MLV RNA and cellular DNA. They are similar to those obtained with AMV and RSV (except that hybridization yields of RNA from both avian viruses appeared to be higher with DNA from Chicken cells than with DNA from mammalian cells) and raise the same questions.

Although only hmw RNA was used annealing might involve cellular RNA still associated to the viral RNA. Previous experiments had shown that the saturation curve of DNA annealed with AMV RNA differed from that obtained with ribosomal RNA. Saturation was not quite achieved when the proportion of bound viral RNA exceeded 0.03 % of the DNA. Also, in contrast with RNA from normal cells, unlabeled AMV RNA, strongly competed for annealing, with labeled AMV RNA (Harel et al., 1966 b). So far, similar experiments could not be performed with sufficient amounts of MLV RNA. However, rough estimations (Huppert et al.), have shown that the specific radioactivity of MLV RNA was comparable to that of AMV or RSV RNAs. Using equivalent inputs, hybridization yields of MLV RNA with Mouse DNA were similar to those of avian virus RNAs with Chicken DNA, and as little affected by competition with cellular RNA.

It is therefore likely that cellular RNA (at least bulk RNA) is not involved in hybridization, and that the proportion of DNA complementary sites is respectively similar for RNAs from both avian and murine viruses.

The peculiar base composition of hybridized MLV RNA (similar to that of annealed AMV or RSV RNAs) may not be attributed to mere RNase degradation for several reasons : It differs from the base composition of the viral RNA "core" after RNase treatment in the same conditions, and does not vary much using too distinct enzymes. It differs also strikingly from the base composition of annealed ribosomal RNA, determined by Attardi et al. (1964) and which is in agreement with our own preliminary data. According to previous work, hybridized RSV RNA sedimented as a single peak of 16 - 18S (Harel et al., 1966 a). In more rigorous conditions which seem to exclude RNA DNA or RNA RNA complexes, the S value of annealed MLV RNA is still higher than that of an RNA "core" alone. The same rigorous conditions were also applied to annealed AMV RNA which sedimented as a 13 - 15S peak (Harel et al. in preparation). This discrepancy in the S value of annealed RNA from different viruses is at the moment of dubious significance.

In conclusion relatively "heavy" fractions of RNA from oncogenic viruses, both avian and murine, appear to be complementary to repetitive sites of DNA from animal cells (either normal or transformed) containing poly T (thymidylic acid) sequences or numerous T clusters. At the moment the biological implications of partial homology between viral and cellular genomes are unknown. It might be due to a fortuitous community of sequences. However, since no such homology could be demonstrated with RNA from two distinct infectious viruses (Harel et al. 1966 b), it is possible to assume that it is restricted to oncogenic (and perhaps related) viruses and may be involved in cell transformation and, or viral replication.

Note : Preliminary data from another laboratory (Galibert, F., Larsen. M., and Boiron. M., Personnal communication) seem to agree with our results.

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