PRESENCE OF COMPLEMENTARY RNA IN CHICKEN SARCOMA CELLS AND ROUS SARCOMA VIRUS

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The subcellular localization of nucleotide sequences in Rous chicken sarcoma complementary to RNA of Rous sarcoma virus was investigated by RNA-RNA molecular hybridization. Samples of virion RNA labeled with radioactive iodine (^{125}I) were annealed with RNA from different fractions (nuclei, mitochondria, free and membrane-bound polysomes) isolated from Rous chicken sarcoma cells. The formation of RNase-resistant hybrids between virus RNA-^{125}I and RNA from mitochondria and membrane-bound polysomes was demonstrated; the relative frequency of occurrence of sequences complementary to virion RNA in the latter, moreover, was 446 times greater than in the former. The role of complementary ribonucleotide sequences is discussed.

KEY WORDS: ribunucleic acids; complementation; subcellular fractions; Rous sarcoma virus; Rous chicken sarcoma.

The synthesis of RNA molecules by purified virions of Rous sarcoma virus (RSV) and avian myeloblastosis virus was demonstrated previously [1], and it led to the suggestion that they have RNA-synthesizing activity. One way of detecting the product of such activity in infected cells is RNA-RNA molecular hybridization. However, very few complementation tests have been performed on cellular and virus RNA by means of this direct method [4, 13].

In this investigation an attempt was made to discover possible homology between RNA preparations isolated from Rous chicken sarcoma cells and RNA from RSV virions (Carr–Zil'ber strain) by the RNA-RNA hybridization method.

EXPERIMENTAL METHOD

RNA was isolated from RSV by the method of Wright and Neiman [15]. The purified RNA preparation was iodinated with ^{125}I by the writers' own method [2]. Tissue from a Rous sarcoma induced in day-old leukemia-free chicks of the S/O phenotype, was isolated 10 days after inoculation. The nuclei were isolated by the method of Chaveau et al. [5], mitochondria were treated with digitonin by Malkin's method [10], and the free and membrane-bound polysomes were purified as described in [3]. RNA was extracted from the preparations of subcellular fractions thus obtained: from the nuclei by the method of Scherrer and Darnell [12], from the mitochondria and both types of polysomes after Perry et al. [11]. ^{125}I-labeled RSV RNA (specific activity $8 \cdot 10^6$ cpm/μg) was sonicated before hybridization on an MSE ultrasonic disintegrator (England) for 6 min (1.5 min each time, 4 times, at 0°C). Hybridization was carried out in a solution containing 0.15 M NaCl and 0.015 M Na citrate (1× SSC), 40% freshly distilled formamide, and 0.2% sodium dodecylsulfate, in sealed capillary tubes (50-100 μl) at 37°C for 24 h [8]. Before incubation the capillary tubes with the samples were heated to 100°C for 10 min and then cooled rapidly to 0°C. After the end of hybridization the samples were cooled on ice, diluted with 1× SSC, treated with RNases A (50 μg/ml) and T_1 (10 units/ml) for 1 h at 37°C, and precipitated with 10% TCA in the presence of bovine serum albumin in the cold. The residues were applied to nitrocellulose filters (0.22 μ). The washed and dried filters were counted in a gamma-counter (VAV-100, East Germany). In each case the activity of the RNases was verified. Only active enzyme preparations were used in the experiments. In all cases when the percentage of RNase-resistant radioactivity was determined, a deduction was made for self-annealing of RSV RNA-^{125}I taking place under the above-mentioned conditions, amounting on average to 7.4%. In some experiments RNA was isolated from mitochondria and membrane-bound polysomes of male C3HA mouse liver and from normal chick embryos.

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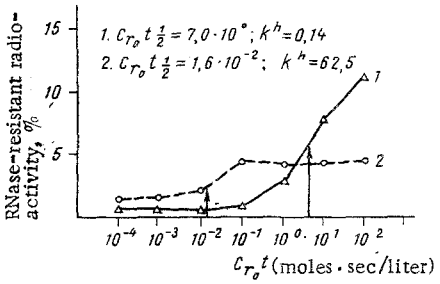

Fig. 1. Kinetics of hybridization of RNA-^{125}I from RSV virions and RNA from mitochondria (1) and membrane-bound polysomes (2) of Rous chicken sarcoma cells. Arrows indicate points corresponding to values of $Cr_0t_{1/2}$.

TABLE 1. Hybridization of RSV RNA-^{125}I with RNA from Subcellular Fractions of Normal and Tumor Tissues

Source of tissue	Level of hybridization, %	
	mitochondria	membrane-bound polysomes
Rous sarcoma	11,8 (11,5—12,0)	4,8 (4,6—4,9)
Normal chick embryos	2,2 (2,0—2,3)	1,2 (1,0—1,3)
C3HA mouse liver	2,1 (2,0—2,3)	0,8 (0,7—0,9)

Legend. 1. Mean values of five experiments shown. 2. All percentages given in Table after deduction of self-annealing of virion RNA, amounting to 7.4% (7.0-7.7). 3. Minimal and maximal values of hybridization shown in parentheses.

EXPERIMENTAL RESULTS

Preliminary experiments showed that RNase-resistant hybrid forms are produced only between virion RNA-^{125}I and ribonucleotide sequences of mitochondria and membrane-bound polysomes from Rous sarcoma. The kinetics of hybridization of these preparations is illustrated in Fig. 1. Clearly hybridization with mitochondrial RNA began at $Cr_0t = 10^{-1}$ (Cr_0t is the product of the initial concentration of nonradioactive RNA and the hybridization time, expressed in moles · sec · liters^{-1}), and the quantity of RNase-resistant radioactivity increases up to $Cr_0t = 10^2$. Meanwhile hybridization with RNA from membrane-bound polysomes began much sooner (between $Cr_0t = 10^{-3}$ and 10^{-2}) and reached a plateau at $Cr_0t = 10^{-1}$.

It is interesting to note that the ratio between the values of k^h (the velocity constant of the RNA-RNA hybridization reaction, equal to $1/Cr_0t_{1/2}$; values of k^h are shown in Fig. 1) is 446. This indicates that the relative frequency of occurrence of sequences complementary to virion RNA in membrane-bound polysomes is 446 times greater than in mitochondria.

To determine the degree of specificity of hybrid formation, RSV RNA-^{125}I was hybridized with RNA from mitochondria and membrane-bound polysomes of normal C3HA mouse liver (heterologous tissue) and from normal chick embryos (homologous tissue). Experiments were carried out in concentrations saturating the virion RNA, i.e., with a 10^5-fold excess of nonradioactive RNA (Table 1). The results of these experiments demonstrated conclusively the high specificity of binding of nucleic acids of Rous sarcoma and RSV.

The presence of sequences complementary to virion RNA in RNA from membrane-bound polysomes can be explained by the existence of an RNA-dependent RNA-polymerase in this fraction. This hypothesis is confirmed by data obtained for microsomes [7], for the method of isolation of the microsomal fraction described in [7] was virtually identical with the method used in the present experiments to isolate membrane-bound polysomes.

The results of hybridization of virion RNA with mitochondrial RNA from tumor cells are particularly interesting. It can be concluded from the results that among the population of mitochondrial RNA there are 11-12% of sequences complementary to virion ribonucleotide sequences. However, it is difficult on the basis of these experiments alone to assess the true representativeness of virus-specific information in the tumor cells.

The ability of the virion and mitochondrial nucleic acids to undergo hybridization can be explained by the presence of RNA-synthesizing activity in the oncornavirus virion itself [1] and by the presence of virus-like particles of oncornaviruses in the mitochondria of tumor cells [9], on the one hand, and by the existence of an RNA-synthetase in the mitochondria themselves, on the other hand. No definite conclusion one way or the other can be drawn from these results.

However, the possibility cannot be ruled out that the hybridization sites in the mitochondria and membrane-bound polysomes are homopolynucleotide sequences and (or) sites of transfer RNA, which are known to be primers for the reverse transcription reaction [6]. The latter hypothesis is supported by the results of experimental hybridization of preparations of RSV RNA-^{125}I with RNA isolated from mitochondria and membrane-bound polysomes of leucosis-free chick embryos (homologous tissue) or from normal mouse liver (heterologous tissue). These sites may also be the connecting links between the subunits of 70S virion RNA [14]. However, further investigation of this problem is necessary to provide the answer to these questions.

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