4. M. B. Davidson and S. A. Kaplan, J. Clin. Invest., 59, 22 (1977).
5. J. D. Goldfine, C. R. Kahn, and D. M. Neville, Biochem. Biophys. Res. Commun., 53, 582 (1973).
6. M. K. Gould, Trends Biochem. Sci., 4, 10 (1979).
7. C. R. Kahn, D. M. Neville, S. P. Gorden, et al., Biochem. Biophys. Res. Commun., 77, 203 (1977).
8. C. R. Kahn, D. M. Neville, and J. Roth, J. Biol. Chem., 248, 244 (1973).
9. K. Masato, A. Yasuo, N. Yasuhiko, et al., Am. J. Physiol., 233, 175 (1978).
10. T. H. Maugh, Science, 193, 220 (1976).
11. D. M. Neville, Biochim. Biophys. Acta, 154, 540 (1968).
12. D. M. Neville, In Vitro, 9, 445 (1974).
13. K. T. Yu and M. K. Gould, Biochem. Biophys. Res. Commun., 77, 203 (1977).

HYBRIDIZATION PROPERTIES OF 4S RNA OF INFLUENZA VIRIONS

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Much experimental evidence has now been obtained to show that virions of a number of influenza viruses contain fractions of low-molecular-weight RNA (lmwRNA) which play an important role in virus reproduction processes [3]. In virions of influenza serotype A the writers discovered an lmwRNA with sedimentation constant of 4S and studied its priming properties in a cell-free reverse transcription system [2].

In the investigation described below, a method of molecular hybridization was used to study the nature of the lmwRNA fraction and the possibility of its interaction with the high-molecular-weight RNA (hmwRNA) of the virions.

EXPERIMENTAL METHOD

Accumulation and purification of influenza virus (strain A/Texas/1/77a), extraction of RNA from the virions, its electrophoresis in 8% polyacrylamide gel (PAG), and isolation of the 4S lmwRNA and hmwRNA fractions were carried out as described previously [2]. By electrophoresis of RNA in 8% PAG followed by extraction of individual fractions, 4S RNA and other forms of lmwRNA, including 4.5S, 5S, and so on, can be clearly separated [1]. The 4S RNA of the virions which was investigated was thus an electrophoretically pure fraction. DNA from chick embryonic skin and muscle tissue cells was isolated by the phenol-detergent method, using RNase A (Sigma, USA) and pronase (Ferak, Berlin). RNA was labeled with ^{125}I with the aid of chloramine T [4]. The specific activity of the preparations reached $2 \cdot 10^5$ cpm/μg RNA. Reassociation of DNA and hybridization of ^{125}I-4S RNA with a large excess of cellular DNA and hmwRNA of the virions was carried out as described in [10]. Competitive hybridization of ^{125}I-4S RNA with a large excess of cellular DNA in the presence of unlabeled 4S RNA from chick embryos was carried out in a solution of 0.4 M Na-phosphate buffer, pH 6.8, containing 40% formamide and 0.2% sodium dodecylsulfate [8] at C_0t = 8-10 moles·sec/liter.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that virion 4S lmwRNA formed duplexes with DNA from chick embryonic cells within the range of concentrations of the latter corresponding to $C_0t_{1/2}$ = 8 moles·sec/liter (curve 1). Comparison of curves 1 and 3 shows that DNA sequences hy-

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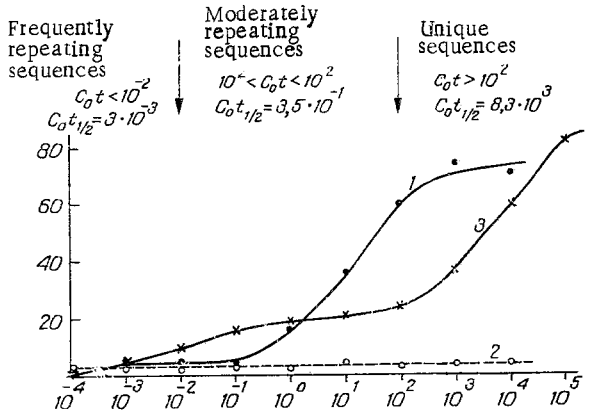

Fig. 1. Kinetics of hybridization of ^{125}I-4S RNA of influenza virions. Abscissa, C_0t (in moles·sec/liter); ordinate, hybridization (in %). 1) Hybridization with DNA of chick embryonic cells, 2) with yeast DNA, 3) reassociation of DNA of embryonic cells. Here and later each point corresponds to mean value of two or three parallel measurements. Level of RNA—DNA hybridization (curve 1) given after deduction of nonspecific hybridization of ^{125}I-4S RNA with yeast DNA (curve 2).

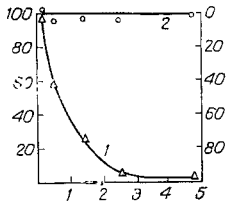

Fig. 2

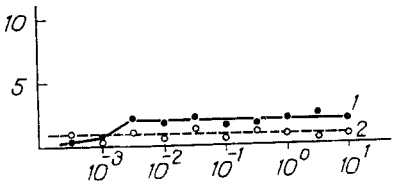

Fig. 3

Fig. 2. Competitive hybridization of influenza virion ^{125}I-4S RNA with chick embryonic DNA in solution. Abscissa, excess of unlabeled RNA (×20); ordinate: left — radioactivity of filters (in %), right — homology (in %). 1) In presence of 4S RNA of uninfected chick embryonic cells, 2) in presence of yeast RNA. Hybridization carried out in the presence of an excess of DNA (C_0t = 10 moles·sec/liter).

Fig. 3. Kinetics of hybridization of influenza virion 4S RNA with excess of hmwRNA in solution. Abscissa, $C_{r_0}t$ (in moles·sec/liter); ordinate, hybridization (in %). 1) Hybridization with influenza virion hmwRNA, 2) hybridization with RNA from Zajdela hepatoma (heterologous control). In all cases, when percentage of radioactivity resistant to the action of ribonucleases was determined, an amount was deducted for self-annealing of ^{125}I-4S RNA, on average 3.3%.

bridizing with virion 4S RNA are included in a group of moderate repeating sequences of the embryonic cell genome. The level of saturation during hybridization of 4S RNA with DNA reached 70% (curve 1), i.e., the highest possible level under the experimental conditions used [9]; this indicates that the virion 4S RNA does not contain nucleotide sequences not present in the cell DNA. This is a strong argument against the 4S RNA originating by degradation of hmwRNA from the virion genome. The high level of saturation during RNA—DNA

hybridization is also evidence of hybrid formation. This conclusion is confirmed by the considerable melting point (T_m of DNA–DNA duplexes was 80°C) (T_m of DNA–DNA duplexes was 87°C). Since a difference of 1° in the values of T_m corresponds to approximately 1.5% of unpaired or imperfectly sequences [7], up to 90% of all nucleotides of 4S RNA took part in the formation of RNA–DNA hybrids. It can be concluded from the results given in Fig. 1 that the 4S RNA found in influenza viriouns is coded by the genome of the chick embryonic cells in which the virus accumulated.

To detect a homology between nucleotide sequences of 4S RNA of virions and embryonic cells, virion ^{125}I-4S RNA was hybridized with cellular DNA in the presence of increasing concentrations of 4S RNA competitor from uninfected cells (Fig. 2). It should be noted that the cellular 4S RNA fraction consisted mainly of tRNA molecules. In the presence of a near hundred-fold excess of unlabeled RNA, virtually complete elimination of ^{125}I-4S RNA from duplexes which it formed with DNA was observed. The logical conclusion is that tRNA molecules enter the virions during their formation within the cell. It is impossible to decide from the existing data whether this process is accidental or whether it reflects a definite specific character of reproduction of influenza virus and is effected by corresponding molecular mechanisms.

To elucidate the possible role of virion 4S lmwRNA it is important to known whether interaction between it and genome RNA is possible. In the case of oncornaviruses, interaction of this sort is responsible for stabilization of the genome RNA and the formation of a template–timer complex which takes part in the synthesis of the DNA anticopy in the infected cell [5, 6]. This experiment to study hybridization of virion ^{125}I-4S RNA with an excess of hmwRNA, the influenza virus genome RNA, is illustrated in Fig. 3. In the region of values of $C_{r_0}t$ up to 10 moles·sec/liter, no appreciable formation of RNA–RNA duplexes was found (curve 1), evidence of the absence of homologous sequences between the virion 4S RNA and genome RNA. This conclusion also was confirmed by a rather different method of recording RNA–RNA duplexes: After hybridization, ultracentrifugation was carried out in a 5-20% sucrose concentration gradient. In this case no transfer, even of part of the radioactive label was observed from the 4S RNA region into the region of RNA of higher molecular weight. Consequently, under the conditions studied 4S RNA and genome RNA of the influenza virions did not form a complex, i.e., they do not possess mutually complementary nucleotide regions. This conclusion confirms previous results indicating that influenza virion 4S RNA cannot perform the function of primer on a virion hmwRNA template during synthesis of complementary DNA [2]. It may be hoped that these facts will help to elucidate the functional role of influenza virion 4S RNA.

LITERATURE CITED

1. A. P. Kozlov, A. Z. Plyusnin, V. I. Evtushenko, et al., Mol. Biol., No. 12, 91 (1978).
2. A. Z. Plyusnin, V. G. Konstantinov, and O. K. Kuznetsov, Byull. Éksp. Biol. Med. (1980) (in Press).
3. T. I. Tikhonenko, The Biochemistry of Virus Particles [in Russian], Moscow (1977).
4. Ya. D. Shaposhnikov, Yu. P. Zerov, Yu. F. Bobrov, et al., Bioorg. Chem. 2, 1351 (1976).
5. E. Canaani, K. van der Helm, and P. H. Duesberg, Proc. Natl. Acad. Sci. U.S.A, 72 401 (1973).
6. J. E. Dahlberg, R. C. Sawyer, J. M. Taylor, et al., J. Virol., 13, 1126 (1974).
7. C. Laird, B. McConoughy, and B. McCarthy, Nature, 224, 149 (1969).
8. M. Melli and J. Bishop, J. Mol. Biol., 40, 117 (1969).
9. M. Melli, C. Whitefield, K. Rao, et al., Nature New Biol., 231, 8 (1971).
10. E. A. Ratovitskii (Ratovitski), Ya. D. Shaposhnikov (J. D. Shaposhnikow), V. K. Guber, et al., Neoplasma, 26, 251 (1979).