Proceedings of the
NATIONAL ACADEMY OF SCIENCES

Volume 45 · Number 11 · November 15, 1959

ON THE SIMILARITY OF CHAIN LENGTH OF NUCLEIC ACIDS

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Communicated by John H. Northrop, September 8, 1959

In a communication two years ago, the author suggested that many cellular or viral ribonucleoproteins[1] (RNP) contain one molecule of ribonucleic acid (RNA) per particle, and that the molecular weight of the RNA in RNP particles is probably about 1.7×10^6 in many cases.[2] This suggestion was based on two experimental findings: (1) In the plant and animal viruses so far studied, the average weight of the total RNA is approximately 2×10^6 times the weight of a hydrogen atom per particle of virus.[3] Similar calculations[2] from data available for a class of large cellular RNP particles, 80S particles, from yeast, pea seedlings, and rat liver give a figure[4] of 1.7×10^6. (2) Determination of the molecular weight of the RNA from a rod-shaped virus, tobacco mosaic virus (TMV), consistently gave a value of 1.7×10^6 under a variety of preparative conditions.[5] The second finding has recently been extended to show that molecules of approximately this weight are also present in all the cellular RNA preparations, so far studied. These include diversified sources such as tobacco leaves,[6] mouse brain,[7] nuclei from calf thymus,[8] and microsomal particles from rat liver.[6]

In this communication, molecular weight of the RNA from a spherical virus, turnip yellow mosaic virus (TYMV), is reported. These values reported for the molecular weights of RNA[9] are then compared with those of deoxyribonucleic acids (DNA) from various sources, and the regularity revealed by this comparison is discussed.

TYMV RNA.—The RNA was isolated at 3°C from this spherical plant virus by a modification of the Gierer-Schramm procedure.[10] A highly purified TYMV suspension[11] containing 1.88 mg virus per ml of 0.01 *M* sodium phosphate, pH 7.2, was shaken for three minutes with an equal volume of 80 per cent redistilled phenol in the phosphate buffer. The resulting emulsion was centrifuged to separate the phases. The aqueous phase was removed and subjected to the process once more. The final clear aqueous layer was extracted with three volumes of ether five times, and then bubbled with N_2 to remove the residual ether. The preparation contained 76 per cent of the RNA in the initial suspension of virus.

A typical schlieren pattern, which was obtained immediately after the preparation was made, is shown in Figure 1. In contrast to the reported sedimentation diagrams for the RNA isolated by a similar procedure from TMV[12] and all cellular sources,[6–8] it is seen to contain a single and rather homogeneous component. The

RNA has a molecular weight of about 1.7×10^6. This is in agreement with the weight of RNA per TYMV particle.[13] Hence, there is one molecule of RNA per TYMV particle. The validity of the author's suggestion has, therefore, been shown for a spherical virus, a rod-shaped virus, and microsomal particles.

The present preparation of TYMV RNA is unstable and dissociates even upon storage at 5°C. The same is true of all RNA samples prepared from other sources by a similar procedure. This instability will explain the much lower values of molecular weight which were previously reported by other workers.[14, 15]

DNA from Bacteriophage $\phi X174$.—A very interesting discovery has been recently made by Sinsheimer.[16] The molecular weight of the DNA isolated from bacterial virus ϕX174 is 1.7×10^6, and this value is also the weight of DNA per ϕX particle. It would be surprising if this situation were limited to this bacterial virus alone. In comparison with the results on RNP described above, the suggestion for RNP is generalized to include deoxyribonucleoprotein (DNP) as follows: A RNP or DNP particle containing about 1.7×10^6 gm of nucleic acid per mole very likely has only one molecule of the nucleic acid.

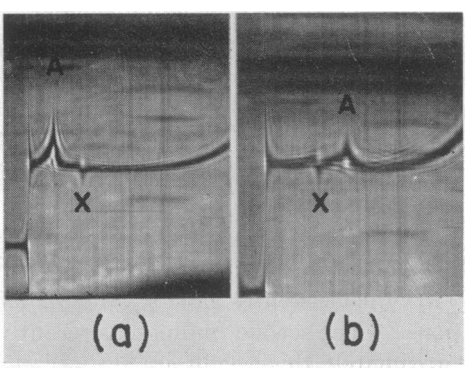

FIG. 1.—Schlieren sedimentation diagram of TYMV RNA (1 mg/ml) in 0.01 *M* sodium phosphate, pH 7.2, at 2°C. Pictures (a) and (b) taken after 4 and 18 min at 59,780 rpm, respectively. The $s_{20,w}$ of the RNA boundary, A, is 28.2S at this concentration (X is caused by a scratch mark on the cell wall).

Cellular DNA.—The DNA preparations from calf thymus, obtained by various procedures, were heterogeneous. The DNA molecule with a molecular weight of 4×10^6 has the highest frequency of occurrence.[17] The analysis on the distribution of molecular weight has not been done yet on the DNA preparations from other sources; however, it has been concluded that the DNA preparations from all diversified sources such as trout sperm, *E. coli*, fowl erythrocyte, and avian tubercle bacilli have the same average molecular weight as that from calf thymus.[18] This conclusion suggests that the DNA which most frequently occurs in many sources other than calf thymus also has a molecular weight of about 4×10^6. Very recently, the same molecular weight has been obtained for a rather homogeneous preparation of transforming principle from pneumococcus.[19]

Results of physicochemical studies indicate that the DNA particles from ϕX174[16] and all cellular[6] and viral[12] RNA so far studied are single-stranded. On the other hand, particles of all other DNA investigated are double-stranded. Hence, the experimental results described indicate that particle weights of about 1.7×10^6 and 4×10^6 have been found at the highest frequency for the single-stranded and double-stranded nucleic acids respectively. In view of the uncertainty involved in the molecular weight values, due to either heterogeneity of the sample studied or approximation of molecular model employed in the calculation, or both, the former figure cannot be considered as different from one-half of the latter. That is, chains of all these nucleic acids appear to have the same length of about 5,000 nucleotides. It does not matter whether the nucleic acid is RNA or DNA. Neither does it

make a difference if the chains are present in the single-stranded or double-stranded form. This has been found true for the preparations from viruses, and from cells of plant, animal, or bacterial origin. The only exception so far is the reported value of 13×10^6 for the molecular weight of DNA from bacteriophage T_4,[20] which is known to have an unusual chemical composition.

In view of the fact that the preparations were obtained with a variety of quite different procedures and that preparations such as transforming principles and viral RNA manifest biological activity, it seems likely that the similarity of chain length described exists among viral and cellular nucleic acids *in situ*.

The term "chain" of nucleic acid used here should not be construed to imply that all these 5,000 nucleotides are linked together only by phosphodiester bonds. The experimental data needed to settle this question are not available at present.

Discussion.—The similarity of chain length suggests that there widely occurs a mechanism in cells for controlling the length of nucleic acid chains. The wide occurrence indicates that it is inadequate to consider the base composition and base sequence of the nucleic acids responsible for the control. One can envisage the existence inside a cell of some kind of "surface" on which the chains are made from nucleotide blocks and by which the chain length is controlled. The larger groove on the two-stranded structure of Watson and Crick for DNA could be a "surface" of this category.[21] At present, however, there is no convincing explanation of why the "surface" should not be of some material other than DNA or why DNA and RNA should use the same kind of "surface."

It is interesting to note that these nucleic acids appear to have a function in common. They are suggested to control the within-cell synthesis of specific viral or cellular proteins. In the case of viral RNA, Fraenkel-Conrat[22] infected the plant with TMV which was made by using the RNA of one strain and the protein of another, and he found that the protein of the viral progeny resembled closely that of the strain from which the RNA was taken but had little resemblance to that of the other parental strain. Furthermore, evidence has been presented that RNA from many plant and animal viruses are infectious,[23] although in the case of the animal viruses much of the evidence is weak owing to lack of proper controls in the experiments. That DNA from the ϕX virus is infectious has not been demonstrated. There is little doubt, however, that the nucleic acid of all viruses plays a genetic role similar to that in TMV.

As to the cellular nucleic acids, the RNA in microsomal particles has been widely inferred to be templates for protein synthesis in the cytoplasm. It is likely that the DNA of human spermatozoa controls, at least in part, the amino acid sequence of haemoglobin.[24] Transforming principles from pneumococcus control the syntheses of the enzymes, mannitol phosphate dehydrogenase,[25] and amylomaltase.[26] Our knowledge about nucleus RNA in this respect is lacking. It will be interesting to see whether the high molecular weight nucleus RNA acts as templates for the protein synthesis in the nucleus, or as the precursor of the high molecular weight cytoplasmic RNA, or as both.

[1] The term nucleoprotein is used throughout the text to denote also particles containing substances in addition to nucleic acid and protein.

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[3] Frisch-Niggemeyer, W., *Nature*, **178**, 308 (1956).

[4] This figure was recently given for 70S particles from *E. coli* also. Tissieres, A., and J. D. Watson, *Nature*, **182**, 778 (1958).

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[6] Gierer, A., *Zeit. Naturf.*, **13B**, 788 (1958).

[7] Cheng, P., *Biochem. Biophys. Acta* (in press).

[8] Cheng, P., *Nature*, **184**, 190 (1959).

[9] *Soluble* RNA will not concern us here.

[10] Gierer, A., and G. Schramm, *Nature*, **177**, 702 (1956).

[11] Was kindly supplied by Professor C. A. Knight of Virus Laboratories at the University of California, Berkeley.

[12] Gierer, A., *Zeit. Naturf.*, **13B**, 477 (1958).

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[16] Sinsheimer, R. L., *J. Mol. Biol.*, **1**, 43 (1959).

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[18] Sadron, Ch., J. Pouyet, and R. Vendrely, *Nature*, **179**, 263 (1957).

[19] Roger, M., personal communication.

[20] Meselson, M., *Cold Spring Harbor Symposia Quant. Biol.*, **23**, 11 (1958).

[21] Cf. Felsenfeld, G., D. R. Davies, and A. Rich, *J. Am. Chem. Soc.*, **79**, 2023 (1957).

[22] Fraenkel-Conrat, H., *J. Amer. Chem. Soc.*, **78**, 882 (1956).

[23] For instance, reference 10.

[24] Ingram, V. M., *Nature*, **180**, 326 (1957).

[25] Marmur, J., and R. D. Hotchkiss, *J. Biol. Chem.*, **214**, 383 (1955).

[26] Lacks, S., and R. D. Hotchkiss, personal communication.

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*THE EFFECT OF HYDRODYNAMIC SHEAR ON THE DEOXYRIBONUCLEIC ACID FROM T_2 AND T_4 BACTERIOPHAGES**

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Communicated by Francis O. Schmitt, September 28, 1959

In 1956, Levinthal[1] reported autoradiographic studies of T_2 and T_4 bacteriophage deoxyribonucleic acid (DNA). He found that the DNA in each phage particle was present in the form of several chains, one of molecular weight about 45×10^6 and six or more of molecular weight about 12×10^6. Attempts to differentiate the large and the small pieces of phage DNA by ultracentrifugation have been unsuccessful. For example, in sedimentation velocity experiments Fleischman[2] found a single peak with a sedimentation coefficient of 30 to 35 S(vedbergs) (the lower value was found on deproteinized preparations); Meselson, Stahl, and Vinograd[3] reported the DNA to band with a Gaussian distribution, implying a uniform molecular weight, in equilibrium experiments in a cesium chloride gradient.

With the ultraviolet absorption system in the Spinco Model E ultracentrifuge, DNA solutions below 0.001 per cent concentration can be studied, and sedimentation coefficients ranging from 8–50 S have been reported.[4, 5] Calculating from the formula which Doty, McGill, and Rice[6] derived from studies on calf thymus DNA, the large and the small pieces of phage DNA could have sedimentation coefficients about 42 and 26 S. These values may not be accurate since they are deduced from an unwarranted extrapolation of the formula, and, moreover, recent studies have