THE REPLICATION PROCESS OF SINGLE STRANDED DNA OF BACTERIOPHAGE φX174

II. THE NON-INTERMEDIATION OF THE DOUBLE STRANDED DNA AS A MATERIAL PRECURSOR

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The mode of replication of the single stranded DNA (single DNA) of bacteriophage φx174 (Sinsheimer, 1959) is one of the interesting problems in the study of nucleic acid synthesis. Sinsheimer et al (1962) have observed that the infecting DNA transforms itself into another type of polynucleotide called the "Replicative form" which replicates and possesses the characters of double stranded DNA (double DNA). In accord with this observation, it has been shown by the isotope tracer technique that the φx174-infected organism synthesizes double DNA prior to (and throughout) the production of single DNA (Matsubara et al., 1963). Several lines of evidence suggest that double DNA plays some role in the production of single DNA. Thus it seemed of interest to determine whether single DNA is synthesized via double DNA since if double DNA is a precursor for the single DNA of the virus, additional mechanisms must exist to separate the two polynucleotide chains and subsequently select the "phage DNA strand". The test was carried out by kinetic studies of the flow of C^{14}-labeled thymine into the two types of polynucleotide. Double DNA was found not to be the immediate precursor for single DNA synthesis.

CALCULATIONS It is known (Matsubara et al., 1963) that φx174 DNA can not replicate when the host is thymine-deficient, and that in infected organisms the synthesis of double DNA starts shortly after infection and continues for 24 min when the cells burst. About 30 φx174 units* are produced per bacterium

per min. Synthesis of single DNA starts at about 9 min and gradually speeds up. A constant rate of synthesis of 100 φx174 units per bacterium per min is attained after 12 min. The late stage of phage growth therefore nearly fits the condition for steady state synthesis.

If it is assumed that thymine molecules flow into single DNA without the intermediation of double DNA, viz. Medium --> Low molecular precursor --> Single DNA, where "Low molecular precursor" represents acid soluble material, and it is further assumed that the size of the precursor pool is constant, and that the reverse reaction does not take place, the kinetics of flow of radioactive thymine may be represented as:

$$\frac{dP^*}{dt} = \frac{M^*}{M}\alpha - \frac{P^*}{P}\alpha \quad \ldots\ldots (1) \qquad \frac{dS^*}{dt} = \frac{P^*}{P}\alpha \quad \ldots\ldots (2)$$

where P,M,S represent the amount of thymine in low molecular precursor, medium and single DNA respectively, P^*,M^*,S^* represent the amount of radioactive thymine in these materials at a time t after the addition of radioactive thymine, and α represents the rate of flow of thymine molecules in the steady state.

A simple calculation from equations (1) and (2) gives S^* to be

$$S^* = \frac{M^*}{M}\left[\alpha t - P\left\{1 - \exp\left(-\frac{\alpha}{P}t\right)\right\}\right] \quad \ldots\ldots (3)$$

Similarly, the same assumptions predict the kinetics of "chase experiments", that is the flow of radioactive thymine after the addition of excess cold thymine ("chaser"). Thus an equation

$$S^* = S_o^* + P_o^*\left\{1 - \exp\left(-\frac{\alpha}{P}t\right)\right\} \quad \ldots\ldots (4)$$

is obtained where t is the time after addition of "chaser", and P_o^*, S_o^* represents the amount of radioactive thymine at t = 0.

The validity of the assumption of non-intermediation of double DNA is demonstrated in a later section.

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* One φx174 unit represents 3.55×10^{-13} μg of thymine, the amount contained in a phage particle.

EXPERIMENTAL Details of the experimental procedures have been described previously (Matsubara et al., 1963). Log phase Escherichia coli 15T⁻D3, a φx174-sensitive derivative of a thymine-requiring 15T⁻ strain, were infected with φx174 at high multiplicity so that all the cells produced phage.

"Pulse" Incorporation Fig. 1 illustrates the synthesis of two kinds of DNA as measured in parallel experiments of short time incorporation (Fig. 2), by adding thymine at 7 min after infection. The rate of single DNA synthesis and the amount of double DNA accumulated after infection was estimated to be, in φx174 units per bacterium, 100 per min and about 1000 respectively. In the next experiment C^{14}-thymine was added at 18 min after infection (t = 0) and incorporation of radioactivity into the two kinds of DNA was followed. The results are shown in Fig. 2.

FIG. 1

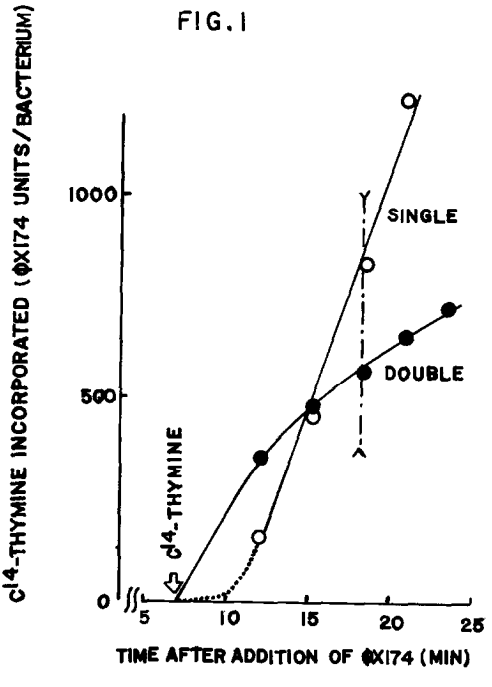

FIG. 2

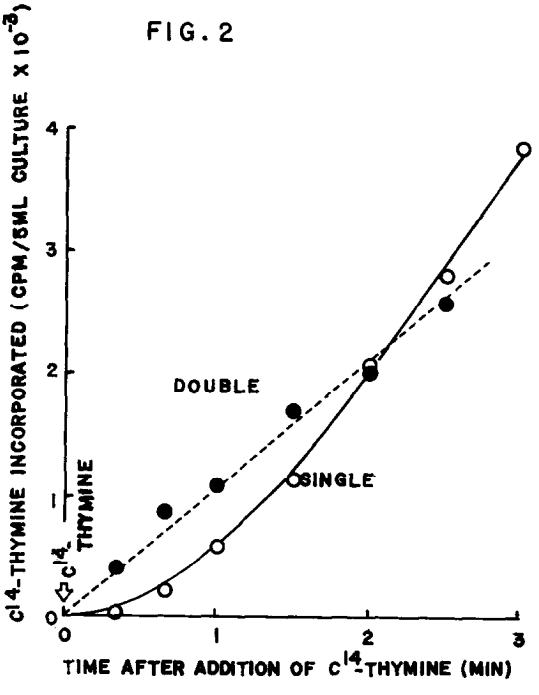

Fig. 1. Synthesis of double and single DNA in E. coli 15T⁻D3 infected with φx174. For experimental details, see legend to Fig. 2.

Fig. 2. "Pulse" incorporation of C^{14}-thymine into double and single DNA.

Five ml of E. coli 15T⁻D3 at 2.0 x 10^8 cells / ml were infected with purified φx174 at an input multiplicity of 15 phages per bacterium in a synthetic

medium. Phage- producing bacteria and surviving colony formers were 99 % and less than 0.5 % respectively of the original colony formers. 2-C^{14}-thymine was added at 18 min after infection (t = 0, 1.33 x 10^5 cpm and 2.3 μg thymine per ml of final culture medium). The reaction was stopped with KCN and immediate chilling of the culture in a dry ice-acetone bath. An aliquot was removed from the chilled culture to obtain the total amount of C^{14} incorporated into acid-insoluble products

Nucleic acid was isolated from the remainder of the chilled culture and subjected to zone electrophoresis to separate double and single DNA (Matsubara et al., 1963). The amount of radioactive thymine incorporated into each kind of DNA was calculated by multiplying the total amount of acid-insoluble C^{14} with the fraction of C^{14} in the respective DNA fraction as obtained from the electropherogram. The solid line is the theoretical curve calculated from equation (3) with $\alpha = 100$ and $P = 133$.

Radioactivity appears without delay in double DNA while a considerable lag occurs before the incorporation of thymine into single DNA, and once started the rate of incorporation into single DNA gradually increases. Therefore it is clear that single DNA is associated with a relatively large-sized precursor pool in contrast to the much smaller pool of double DNA precursor.

By extrapolating the curve of incorporation into single DNA to t = 0, the size of precursor pool was estimated to be about 130 φx174 units per bacterium.

"Chase" Experiment If double DNA is the immediate precursor for single DNA, the label in the former should flow into single DNA in an appreciable amount when "chaser" is added since the precursor pool for double DNA is small.

As shown in Fig. 3, the incorporation of radioactive thymine into double DNA was stopped immediately on addition of the "chaser". The level of radioactivity in the DNA remained constant for about 2 min and then gradually decreased. On the other hand, radioactivity continued to flow into single DNA for about 5 minutes after addition of the "chaser", exceeding the amount of radioactivity released from double DNA.

It is clear, therefore, that double DNA is not the immediate precursor for single DNA. The slow decrease of radioactivity in the former may represent the gradual non-specific breakdown of DNA in the infected complex.

The solid lines in Figs. 2 and 3 represent the theoretical curves for incorporation of radioactivity into single DNA as described above with values for α

of 100 and for P of 133. These curves fit the observed data showing that the results are, in principle, interpretable by the simple hypothesis stated above.

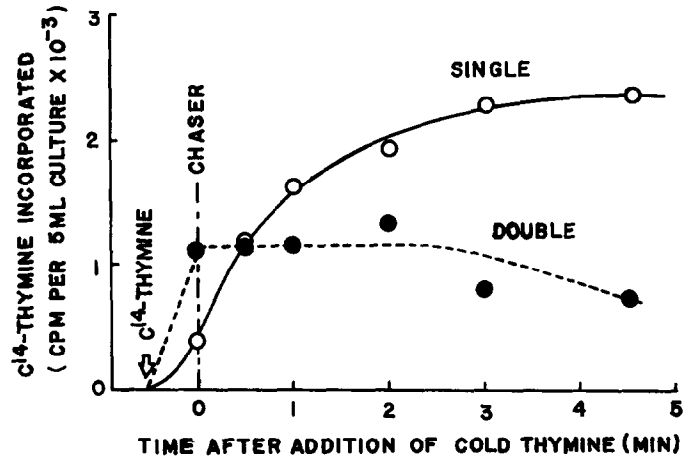

Fig. 3. The kinetics of C^{14}-thymine incorporation into double and single DNA after addition of "chaser".

Labeled thymine was added at 18 min after infection (3.14×10^5 cpm and 2.3 μg of thymine per ml of final culture medium) and after an additional 30 seconds, 120 times the amount of cold thymine was added (t = 0). The subsequent procedures were the same as described in the legend to Fig. 2. The solid line is the theoretical curve calculated from equation (4) with $\alpha = 100$ and P = 133.

DISCUSSION The relationship of the two DNA's in a φx174-infected organism may be summarized as follows:

1. The double DNA that accumulates after infection is not the immediate precursor for single DNA.
2. Single DNA is associated with a relatively large-sized, low molecular weight precursor pool, while double DNA is not. The low molecular weight precursor is represented here by acid-soluble materials containing thymine.
3. The size of the precursor pool for single DNA is estimated to be about 130 φx174 units per bacterium.

The nature of the apparent differentiation in thymine-containing precursors for the synthesis of double and single DNA awaits further elucidation.

The present idea that the double DNA synthesized and accumulated in the cell after infection is not the immediate precursor for single DNA does not exclude the possibility that some type of double DNA plays a role as the precursor for single DNA. However, such a hypothesis must include the assumption of an exceedingly rapid turnover of the double DNA precursor or the assumption of the degradation of the DNA to an acid soluble product by the commonly employed treatment with cold acid.

To account for the close relationships between the synthesis of double and single DNA in an infected organism, it is tempting to assume that double DNA carries the information necessary for the synthesis of single DNA.

The double DNA may replicate itself through complementary synthesis (Watson and Crick, 1953) and then serve as template for another type of DNA-synthesizing mechanism which develops after infection (Matsubara et al., 1963). This hypothesis fits the observation (Kozinski, 1961) that parental ϕx174 DNA can not be transferred to the progeny DNA as an integral unit, since transformation of parental DNA to the double stranded form may make its reversion to the single stranded form impossible. The situation thus resembles the role of bacterial DNA in the synthesis of cellular ribonucleic acids.

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