ON THE REPLICATION OF SINGLE-STRANDED DNA OF BACTERIOPHAGE ØX 174

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Sinsheimer et al.(1) have studied the replication process of the single-stranded DNA of bacteriophage ØX 174. Their findings show that the synthesis of new single-stranded DNA takes place through a replicative form which possesses the characters of double-stranded DNA. The manner of formation of the ultimate single-stranded DNA progeny from double-stranded replicative form remains obscure. Whether the double-stranded DNA is the immediate precursor of the single-stranded DNA or not is also an open question.

The studies on kinetics of this synthesis by Matsubara et al. (2) have certainly helped, at least, in suggesting some reasonable hypotheses with respect to the exact role played by the double-stranded DNA. They have indicated that the double-stranded DNA is not the immediate precursor, though it does carry the information necessary for the synthesis of single-stranded DNA. Singh (3) has pointed out that the findings of Matsubara et al. do not exclude the possibility that double-stranded DNA is the immediate precursor.

If the double-stranded DNA is the immediate precursor of the single-stranded DNA as Singh has suggested, the scheme of passage of

thymine in the experiment of Matsubara et al. would be:

MEDIUM —> LOW MOLECULAR PRECURSOR —>

—> DOUBLE-STRANDED DNA —> SINGLE-STRANDED DNA

If M, P, D, and S respectively represent the amount of thymine in these materials after time "t" and M^*, P^*, D^* and S^* are the respective amounts of radioactive thymine after "t", then the dynamic equations of the process would be:

$$\frac{dS^*}{dt} = \frac{D^*}{D} a \tag{1}$$

$$\frac{dD^*}{dt} = \frac{P^*}{P} a - \frac{D^*}{D} a \tag{2}$$

$$\frac{dP^*}{dt} = \frac{M^*}{M} a - \frac{P^*}{P} a \tag{3}$$

where a is the rate of flow of thymine in the steady state. The solutions of these equations and relevant substitutions give us:

$$S^* = \frac{M^*}{M}\left[at - P\left(1 - e^{-\frac{at}{P}}\right) - D\left(1 - \frac{P}{P-D} e^{-\frac{at}{P}} + \frac{D}{P-D} e^{-\frac{at}{D}}\right)\right] \tag{4}$$

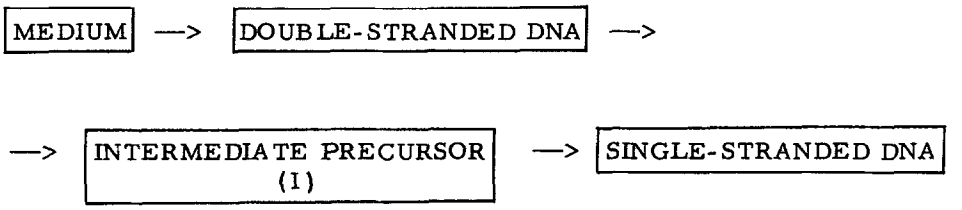
which agrees with the experimental curve (Matsubara et al., Fig. 2).

D^* is now given by:

$$D^* = \frac{M^*}{M} D\left(1 - \frac{P}{P-D} e^{-\frac{at}{P}} + \frac{D}{P-D} e^{-\frac{at}{D}}\right) \tag{5}$$

which is an exponential relation between D^* and t and not a straight line relation as observed by Matsubara et al.

If now the scheme for passage of thymine is assumed to be as follows:



where the intermediate precursor (I) can be the acid soluble material hinted at by Matsubara et al., then the dynamic equations become:

$$\frac{dS^*}{dt} = \frac{I^*}{I}a \tag{6}$$

$$\frac{dI^*}{dt} = \frac{D^*}{D}a - \frac{I^*}{I}a \tag{7}$$

and

$$\frac{dD^*}{dt} = \frac{M^*}{M}a - \frac{D^*}{D}a \tag{8}$$

Equations 6, 7 and 8 give:

$$S^* = \frac{M^*}{M}\left[at - D\left(1 - e^{-\frac{at}{D}}\right) - I\left(1 - \frac{D}{D-I}e^{-\frac{at}{D}} + \frac{I}{D-I}e^{-\frac{at}{I}}\right)\right] \tag{9}$$

which once again fits the experimental curve. Further it gives:

$$D^* = \frac{M^*}{M}D\left(1 - e^{-\frac{at}{D}}\right) \tag{10}$$

which is also an exponential relation. However, on expansion, for small values of $\frac{at}{D}$ it does give a straight line passing through the origin as observed experimentally by Matsubara et al.

The values obtained for S^* and D^* in the foregoing are valid only under the following assumptions:

a) In the steady state, the rate of flow of thymine is constant and is the same for all the individual intermediate steps in the scheme.

b) Rate of flow of thymine in steady state (α) is much less than the total amount of thymine in the double-stranded DNA (D), i.e., $\alpha \ll D$.

c) All the measurements are made in the steady state.

d) Amount of radioactive thymine taken up from the medium is random and truly reflects the net DNA synthesis.

Thus it would seem that the double-stranded DNA may not be the immediate precursor of the single-stranded DNA of bacteriophage ØX 174. However, it is possible that it may trigger the synthesis of the single-stranded DNA, through an intermediate (I) like the one mentioned above.

REFERENCES

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3. Singh, U. N., Biochem. Biophys. Res. Commun. 15, 220 (1964).