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Computer simulation of T3 / T7 phage infection using lag times

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A minimal mechanism is proposed which describes the transcriptional and translational processes for four phage proteins (RNA polymerase, DNase, primase and DNA polymerase) involved in T3/T7 DNA replication. Phage DNA replication is also included. It is shown how lag times may be incorporated into a kinetic mechanism. The distinct three-stage transport of phage DNA into the bacterial host (*E. coli*) is considered. DNA transport is assumed to be rate-determining for the transcription of class I and II proteins. Transcriptional and translational lag times have been calculated on the basis of available gene mapping of T7 phages. The kinetic behavior of T7 and T3 phage infection is practically identical. The hydrolysis of bacterial DNA by phage DNase (endonculease and exonuclease) as well as the subsequent phosphorylation to the deoxymononucleoside triphosphates are assumed to be rate-determining in phage DNA replication. Good agreement with experiment is obtained in our computer simulations.

1. Introduction

In an effort to understand better some guiding principles in evolving and living systems [1–3] we have attempted to simulate on a computer the kinetics of protein synthesis and DNA replication in DNA phage infection of *E. coli* bacteria. We have chosen the T7 bacteriophage system since Dunn and Studier [4] have recently finished the determination of the sequence of the entire 39 936 base-pairs of T7 DNA including the positions of the 50 individual genes and the genetic signals. The infection process of T3 and T7 bacteriophage is unique since the phage DNA transport into the bacterium occurs in three distinct stages. Recent work by Zavriev and Shemyakin [5] has improved our quantitative understanding of these processes. Our actual computations are based on experimental work by Hausmann and Härle [6] for T3 bacteriophage, providing the time course of a small number of proteins and T7 DNA during a single infection cycle (fig. 1). All sequence information comes from the T7 phage system. However, it is generally accepted that the kinetics of gene expression is practically identical in T3 and T7 phages with only minor differences. These differences do not concern phage DNA replication [7] which is one of the main interests here. An astonishing fact reveals itself: all proteins, enzymes and the phage DNA are produced sequentially in such a way that each species seems to start practically discontinuously from zero concentration to reach values commensurate with the formation of about 100 bacteriophage particles per infected *E. coli* bacterium (fig. 1).

The abrupt rise of all individual proteins and DNA cannot be modeled by the sole use of rate constants. Instead, we introduce the concept of lag times [8]. It is the aim of this work to show how lag times may arise in the transcription and translation processes in phage and how these lag times may be incorporated into a simple kinetic mecha-

Dedicated to Manfred Eigen on the occasion of his 60th birthday.

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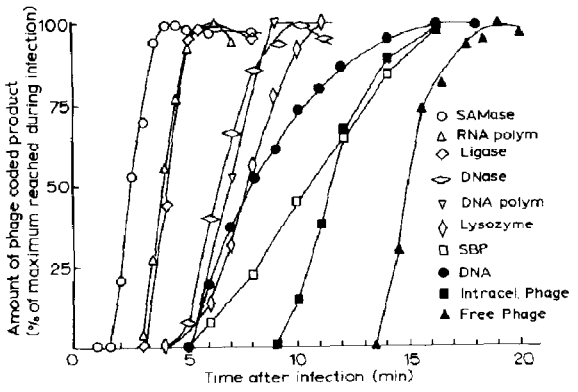

Fig. 1. Synthesis of T3 bacteriophage proteins in *E. coli* at 37°C as a function of time. Note that relative % concentrations are given only. Reproduced from ref. 6 with permission of the authors.

nism that also contains the necessary rate constants which describe the subsequent shape of the kinetic curves. Unfortunately, the absolute concentrations of the individual phage proteins and DNA are not known; instead, relative concentrations normalized to 100% formation of the respective species have been reported only. Therefore, our second-order rate constants represent normalized values which must be multiplied with appropriate factors arising from future determinations of absolute species concentrations. A further aim of this work was to provide a possible distinction between two extreme phage DNA replication mechanisms, namely, an autocatalytic (semiconservative) and a simple template mechanism in which all DNA copies may be produced from one original DNA double strand. It will be seen that our calculations do not distinguish between the two possibilities, since hydrolysis of bacterial DNA, which furnishes the mononucleotides, turns out to be rate-determining in phage DNA replication.

2. The mechanism of infection

The mechanism of infection of T7 bacteriophage and the transcription of its T7 DNA is relatively complex [9,10] and one cannot yet hope to include all elementary steps to simulate all kinetic processes in detail. Therefore, drastic simplifications are necessary which will lead to what we consider a minimal mechanism only. In this first attempt we include only those proteins in our calculations that are important in phage DNA replication [11,12]. The inclusion of all other proteins is straightforward but it unduly increases the complexity of the calculations. T7 bacteriophage infection starts with the adsorption of phages on receptor sites on the membrane of the bacterium. The left end of the double-stranded T7 DNA enters the *E. coli* bacterium within an unspecified short time in order to provide the start for the transcription process carried out by the host RNA polymerase which is present in sufficient concentrations. The uniqueness of T7 (and T3) bacteriophage concerns the three distinct stages at which transcription takes place (fig. 2). In the first stage (class I proteins) T7 DNA transcription occurs at a relatively slow rate which we consider to be constant, starting from three strong promoters at approx. 2% and continuing to the terminus at 19.0% T7 DNA. The rate-determining process in class I transcription is considered to be the relatively slow transport of class I DNA into the bacterium, which is assumed to occur at a constant rate.

The position in T7 DNA is expressed in units of 1% of the length of the DNA molecule and a T7 unit corresponds to approx. 400 base-pairs [4]. One of the main class I proteins is T7 RNA polymerase together with other proteins (a protein kinase and an inhibitor) that inactivate the host RNA polymerase. The newly formed T7 RNA polymerase catalyzes the transcription of the second stage proteins which we calculate to occur at a constant rate higher by about a factor of 4 than that for class I transport. T7 DNA polymerase is one of the class II proteins and it replicates the total DNA of the phage. Further entry of T7 DNA into the bacterium occurs in stage III which is thought to be coupled to the transcription process itself [5,13]. Class III protein synthesis is not considered here, since it mainly concerns the capsid proteins. Some of the later structural proteins (e.g., gene 11 and 12) of the T7 tail are thought to be transcribed by readthrough (fig. 2b) which may

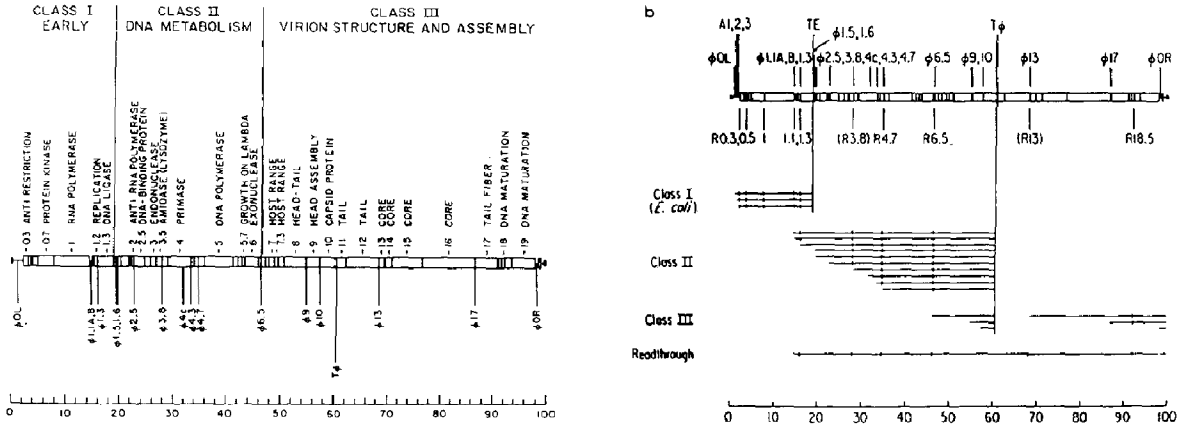

Fig. 2. (a) Genetic map of bacteriophage T7 DNA with a number of proteins. Open boxes refer to the T7 genes with gene numbers. φ are promoter locations and T indicates a terminator for T7 RNA polymerase. Filled boxes refer to terminal repetition, OL and OR indicate left and right end, origin of replication. Reproduced from ref. 4 with permission of the authors. (b) Synthesis of bacteriophage T7 mRNAs. *R* refers to an RNase III cleavage site. Horizontal lines indicate primary transcripts from each promoter and short vertical lines refer to RNase III cleavage sites. Parentheses indicate that not all RNAs are cut. Readthrough of Tφ is also indicated as the 'longest' transcript. Reproduced from ref. 4 with permission of the authors.

provide the optimal concentrations for efficient self-assembly of T7 DNA and capsid proteins to produce intracellular phages. Lysis of the bacterium occurs after approx. 13.5 min under optimal nutrient conditions to produce about 100 T7 phage particles per infected *E. coli* cell.

2.1. Delay times in T7 phage infection

All reaction steps containing a delay time are assumed to be of first order which means that the produced intermediates C_i are delayed from further reactions for the duration of their specified time intervals T_i. This procedure ensures that the products rise from zero concentration in an arbitrarily steep fashion.

2.1.1. Class I proteins

The time for the transcription of gene 1 (T7 RNA polymerase) is denoted by the latency time T_1. An appropriate value for T_1 may be estimated from experiments carried out by Zavriev and Shemyakin [5] who studied the entrance kinetics of T7 DNA into the host. Their data reveal that the rate of transcription is about 1% T7 DNA/11 s. We consider this rate to be constant up to 19.0% T7 DNA which represents the end of the genome for class I proteins. This rate of transcription is considered to be transport-controlled, i.e., it is slower than the rate of free transcription. Thus, the rate of transcription is set equal to the mechanical transport rate of the class I sequence of T7 DNA being injected into the bacterial cell where injection is aided by the host RNA polymerase in an as yet unspecified fashion. Gene 1 (T7 RNA polymerase) is located between 7.94 and 14.57% of the T7 DNA [4]. The bacterial RNA polymerase starts its transcription at the promoters A_1, A_2 and A_3 which are located near the left end of the DNA at the 1.25, 1.57 and 1.88% marks, respectively, and proceeds to the RNase III cleavage site at 14.74% T7 DNA. Therefore, the host RNA polymerase transcribes approx. 12.7% of the T7 DNA. This process takes about 140 s. From the fit to the total experimental delay time for RNA polymerase we obtain 20 amino acids/s for the

rate of translation which will be used throughout the calculations for want of any more direct information. The messenger of T7 RNA polymerase starts at the RNase III cleavage site (R1) which coincides with the left end of the T7 RNA polymerase gene 1. T7 RNA polymerase consists of 883 amino acids; the time for translation is about 44 s. Thus the first molecule of T7 RNA polymerase appears after 184 s as required by the experimental data (fig. 1 and table 1). The shape of the subsequent increase in T7 RNA polymerase concentration is determined by the values of the individual rate constants given later. The last class I protein to be transcribed and translated is ligase (gene 1.3). Its genome is located between 16.21 and 18.91% T7 DNA. Total transcription takes about 187 s to the 19.0% mark (terminus for class I proteins); translation requires about 18 s for the first ligase molecule to be synthesized which should appear after 3.4 min in fair agreement with experiment. In our computer simulations ligase has not been considered, since it is involved only in the later stages of DNA metabolism.

2.1.2. Class II proteins

Transport into the bacterium of class II DNA starts at 19.0% T7 DNA. Since phage RNA polymerase is available, the rate of transport of class II DNA into the bacterium is considered to be higher (1% T7 DNA/2.5 s) than that of class I T7 DNA. T7 RNA polymerase is assumed to aid in the mechanical transport mechanism [5]. This transport rate is derived from the experimental data for T7 DNA polymerase. T7 DNA polymerase transcription must proceed at least to the RNase III cleavage site (R 6.5) at 46.48% T7 DNA. The total transcription time (up to R 6.5) is the sum of class I (187 s) and class II (69 s) transcription. Since an RNase III cleavage site exists at 34.79% (R 4.7), the actual time for translation corresponds to the messenger sequence from 34.79 to 41.23%, the latter being the end point for T7 RNA polymerase. The time required for this translation sequence is about 43 s. Thus, the first T7 DNA polymerase molecule will appear after about 299 s as required by experiment.

Experimental data are available for the formation of the following class II proteins: DNase, lysozyme and T7 DNA polymerase, for which all gene locations are known. Delay times for DNase and lysozyme may be calculated in an analogous fashion. Lysozyme has not been used in our computations, since it is not involved in DNA synthesis. Its future inclusion is expected to be straightforward. On the other hand, we included primase in the simulations for which experimental kinetic data are not available. The experimental delay time for primase is predicted to be 4.3 min on the basis of its genome location and the above DNA transport rates. The role of endonuclease is to initiate the hydrolysis of host DNA to form duplex DNA fragments [14,15]. The position of the endonuclease (gene 3) is 25.68–26.80% T7 DNA units. The overall elapsed time to reach the end point for

Table 1

R, RNase III cleavage site [4]; T_c, total lag time for transcription up to R [4]; T_L, lag time for translation from the previous R to the end of the gene; T_i, lag time for the expected appearance of the protein as calculated from the gene map; T_i(exp), experimentally determined lag time [6] for the appearance of the protein.

Class	Gene	Protein	Gene location on T7 DNA (%)	R (%)	T_c (s)	T_L (s)	T_i (s)	T_i(exp) (min)
I	1	RNApol	7.94–14.57	14.74	140	44	184	3.0
I	1.3	Ligase	16.21–18.91	19.0	187	18	205	3.0
II	3	Endonuclease	25.68–26.80	28.05	210	52	262	4.4 [a]
II	3.5	Lysozyme	26.81–27.94	28.05	210	60	270	4.5
II	4A,4B	Primase	28.96–33.21	34.79	227	34	261	–
II	5	DNApol	35.94–41.23	46.48	256	43	299	5.0
II	6	Exonuclease	43.47–46.08	46.48	256	75	331	–

[a] Equal to DNase of ref. 6.

endonuclease transcription is about 187 s for class I DNA transport up to the RNase III cleavage site at 28.05%. Translation of endonuclease must start at the 18.9% mark, since all proteins encoded between 18.9 and 25.68% have to be translated first. The total time for translation including the previous proteins is 52 s which leads to a total delay time of 4.4 min for endonuclease.

The exonuclease (gene 6) is synthesized later at about 5.5 min according to our calculations (table 1). Exonuclease is believed to hydrolyse the duplex fragments eventually to the 5'-mononucleotides. For the sake of simplicity, in our computer calculations we combine endonuclease and exonuclease into one protein called DNase as measured by Hausmann and Härle [6].

2.2. T7 DNA replication

Experimental evidence shows that the beginning of phage DNA synthesis coincides with the start of DNA polymerase synthesis (fig. 1). In this sense DNA polymerase provides the lag time for DNA synthesis. Therefore, an explicit lag time for phage DNA is not used. Primase is already available to aid in DNA synthesis. Available deoxynucleoside triphosphates of the host are incorporated before further deoxynucleoside triphosphates are supplied via the hydrolysis of host DNA.

2.3. Concatemers

Phage DNA is known to be synthesized as concatemers [17–20] with an average distribution of about 2–3 unit lengths of DNA per concatemer [17]. This feature will not be part of our mechanism, since we simply calculate the total amount of incorporated monomers as a function of time. Concatemer formation is not assumed to be kinetically important under these conditions, since it does not change the concentration of incorporated species. Reinitiation [21] should blur the sequence of DNA 'generations' and lead to a more gradual rise in DNA concentration. This latter feature is observed experimentally and is inherent in our mechanism.

3. A minimal mechanism

We have incorporated these facts into a mechanism which has been numerically integrated. This proposed minimal mechanism contains T7 DNA transcription, translation and T7 DNA replication and it shows how delay times as well as rate constants may be used:

$$\mathrm{RNApol}_{bac} + \mathrm{DNA} \xrightarrow{k_1} \mathrm{C}_1 \tag{1}$$

$$\mathrm{C}_1 + \mathrm{Mp} \xrightarrow{k_2} \mathrm{C}_2 \tag{2}$$

$$\mathrm{C}_2 \xrightarrow{T_1} \mathrm{mRNA}_1 + \mathrm{DNA} \tag{3}$$

$$\mathrm{mRNA}_1 + \mathrm{Me} \xrightarrow{k_3} \mathrm{C}_3 \tag{4}$$

$$\mathrm{C}_3 \xrightarrow{T_2} \mathrm{RNApol} \tag{5}$$

$$\mathrm{RNApol} + \mathrm{DNA} \xrightarrow{k_4} \mathrm{C}_4 \tag{6}$$

$$\mathrm{C}_4 + \mathrm{Mp} \xrightarrow{k_5} \mathrm{C}_5 \tag{7}$$

$$\mathrm{C}_5 \xrightarrow{T_3} \mathrm{mRNA}_2 + \mathrm{DNA} + \mathrm{RNApol} \tag{8}$$

$$\mathrm{mRNA}_2 + \mathrm{Me} \xrightarrow{k_6} \mathrm{C}_6 \tag{9}$$

$$\mathrm{C}_6 \xrightarrow{T_4} \mathrm{DNase} \tag{10}$$

$$\mathrm{C}_6 \xrightarrow{T_5} \mathrm{primase} \tag{11}$$

$$\mathrm{C}_6 \xrightarrow{T_6} \mathrm{DNApol} \tag{12}$$

$$\mathrm{DNase} + \mathrm{DNA}_{bac} \xrightarrow{k_7} \mathrm{Mp}' + \mathrm{DNase} \tag{13}$$

$$\mathrm{Mp}' \xrightarrow{k_8} \mathrm{Mp}'' \tag{14}$$

$$\mathrm{DNApol} + \mathrm{C}_4 \xrightarrow{k_9} \mathrm{C}_7 + \mathrm{RNApol} \tag{15}$$

$$\mathrm{Primase} + \mathrm{C}_7 \xrightarrow{k_{10}} \mathrm{C}_8 \tag{16}$$

$$\mathrm{C}_8 + \mathrm{Mp}'' \xrightarrow{k_{11}} \mathrm{DNA} + \mathrm{DNA}' + \mathrm{DNApol} + \mathrm{primase} \tag{17}$$

The first mechanistic event is the rapid penetration of the first base-pairs of T7 DNA into the

bacterium. The time for this process is considered to be very short compared with most of the subsequent events. For this reason the very first injection event is not considered to be kinetically important in our calculations.

Step 1 is considered to be the rapid formation of a complex between host RNA polymerase and the left promoter region (A_1, A_2 and A_3) of the T7 DNA. This transcription complex (C_1), incorporates nucleoside triphosphate monomers to form a species C_2 which releases messenger RNA ($mRNA_1$) and the original T7 DNA after a specified delay time T_1 (= 2.3 min) has elapsed. The abruptness of this process is simulated by step 3 which indicates that the messenger (contained in C_2) is made suddenly available after a specific delay time has passed. The concentration of $mRNA_1$ is equal to zero before T_1. Thus, the delay time simulates the abrupt rise of mRNA caused by the particular T7 DNA transport mechanism into the bacterial cell. For illustration the kinetic delay equations for $mRNA_1$ may be written as

$$\frac{d[mRNA_1]}{dt} = k_2([C_1](t-T_1))([M_p](t-T_1)) - k_3[mRNA_1][M_e]$$

where M_p and M_e are the ribonucleoside triphosphates and activated amino acids, respectively. It is seen that $mRNA_1$ appears suddenly at T_1.

For example, the rate equation for C_2 is:

$$\frac{d[C_2]}{dt} = k_2[C_1][M_p] - k_2([C_1](t-T_1)) \times([M_p](t-T_1))$$

where the first term resembles the 'normal' rise of C_2 and the second term is a delayed first term to be subtracted from the normal first term. Another important characteristic of steps 1–3 is the irreversible disappearance of the host RNA polymerase which is inhibited in the in vivo system. $mRNA_1$ cannot be translated during the first delay time T_1.

$mRNA_1$ and ribosomes rapidly diffuse together to translate the messenger into RNA polymerase by the uptake of activated amino acids (step 4). The total process happens during the delay time T_2 (= 0.7 min) after which the first T7 RNA polymerase molecule appears. Class II transcription starts with the availability of T7 RNA polymerase and the further continuous transport of T7 DNA into the bacterium. The higher rate of injection of class II T7 DNA (1% DNA/2.5 s) is represented by a shorter delay time T_3 (= 0.7 min) for transcription. We also assume the transport of class II T7 DNA to be rate-determining for transcription. It is noted that T7 DNA and T7 RNA polymerase are completely recovered after transcription as indicated in step 8, whereas all messenger RNA molecules disappear. Step 13 represents the hydrolysis of the host DNA by T7 DNase leading to nucleoside monophosphates (Mp') which are phosphorylated in step 14 to obtain the deoxynucleoside triphosphates (Mp'') necessary for DNA replication. The latter two steps thus provide a new source for the deoxynucleoside triphosphates as reactants. After all of the host DNA has been hydrolysed further replication will stop.

In step 15, T7 RNA polymerase is displaced by T7 DNA polymerase [11] from complex C_4 to form a new complex C_7 that combines with primase [11,16] to form C_8 which reacts further with phosphorylated nucleoside triphosphates (Mp'') to the newly replicated T7 DNA (DNA') and the original DNA (step 17). The reason for the discrete assignment of DNA' as the newly formed T7 DNA is to study the kinetic difference between simple catalytic and autocatalytic growth of T7 DNA. Autocatalysis is formally encountered if T7 DNA is set equal to DNA'. For this case DNA' is 'recycled' through the early steps 1–8 of the mechanism after a total delay time of 5.0 min ($T_1 + T_2 + T_3 + T_6$) has elapsed. Thus, additional C_4 will be produced in step 6 which is a reactant in step 15 and the total T7 DNA concentration increases autocatalytically. If [DNA] ≠ [DNA'], the newly replicated DNA' will not take part in its own replication and subsequent translation processes and its growth will be solely due to a catalyzed copying process of T7 DNA as the original template. In our mechanism further deoxynucleoside triphosphates are provided by hydrolysis of the host DNA and the subsequent phosphorylation of the deoxynucleoside monophosphates.

4. The computations

The results of our computer simulations are shown in fig. 3 for phage RNA polymerase, DNase, DNA polymerase and phage DNA. Primase was also calculated but is not included in fig. 3, since experimental data for primase are not available. An optimal fit has been achieved between experiment and the above mechanism with

$T_1 = 2.3$, $T_2 = 0.7$, $T_3 = 0.7$, $T_4 = 0.7$, $T_5 = 0.8$,
$T_6 = 1.3$, $k_1 = 15$, $k_2 = 1$, $k_3 = 5$, $k_4 = 40$,
$k_5 = 1$, $k_6 = 10$, $k_7 = 0.7$, $k_8 = 0.55$, $k_9 = 100$,
$k_{10} = 100$, $k_{11} = 10$

where all latency times are given in minutes, all first-order rate constants in min^{-1} and all second-order rate constants in (reduced concentration)$^{-1}$ min^{-1}. For class II proteins simplifications had to be introduced in order to keep the calculations tractable. Therefore an average transcriptional delay was used ($T_3 = 0.7$ min) for all class II proteins, DNase, primase and DNA polymerase. However, their individual total delay times are not affected by this simplification. Their respective transcriptional delay times follow from the difference to the experimentally known total delay times (table 1) as $T_4 = 0.7$ min, $T_5 = 0.8$ min and $T_6 = 1.3$ min, respectively. Primase arises after T_5 and helps to replicate T7 DNA in step 16 [22].

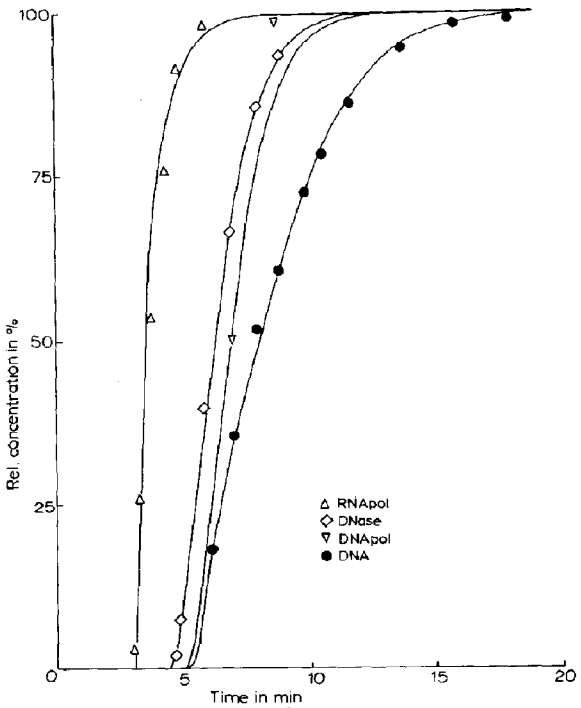

Fig. 3. Computer simulations of our minimal mechanism and experimental data [6] for phage RNA polymerase, DNase, DNA polymerase and phage DNA as a function of time. Primase was calculated but not shown. Note the relatively abrupt rise of the calculated curves as obtained by the use of lag times and required by experiment.

Initial conditions for Mp, Me and Mp″ are 1.0, 2.3 and 0.25, respectively. We assume that T7 DNA polymerase initially incorporates all available deoxynucleoside triphosphates of the host; the rest of the incorporated monomers (75%) are furnished by *E. coli* DNA degradation. The theoretical fits of the template mechanism ([DNA] ≠ [DNA']) and autocatalytic replication ([DNA] = [DNA']) are practically identical. The reason for this similarity turns out to be the fact that the supply of deoxynucleoside triphosphates from host DNA hydrolysis must be rate-determining in order to fit the data. In fact, a slight decrease in slope at about 25% incorporation indicates a 'switch over' from host monomers to the monomers which are furnished in a rate-determining step by hydrolysis of bacterial DNA. Any differences in the above two mechanisms are kinetically indistinguishable. In general, DNA replication, being a semiconservative process, is considered to be autocatalytic as more than one copy is produced. It seems that the minimal mechanism fits the experimental data relatively well for the selected proteins that are important in DNA replication. DNA replication itself is seen to be well simulated. In particular, the fit regarding the lag times is optimal although there are limited data in the neighborhood of zero relative concentrations. The fit becomes unsatisfactory at low reduced concentrations for the limiting case of zero lag times (not shown). For the latter case the initial rise of protein and DNA concentrations would be gradual in disagreement with experiment. The values of the rate constants determine only the subsequent shape of the kinetic curves.

5. Discussion

The above mechanism represents a minimal mechanism whose characteristic feature is the use of lag times. Lag times are necessary for processes with delayed appearance of products such as in simple transcriptional and translational events coupled with transport-controlled DNA injection. The sole use of rate constants would lead to a gradual appearance of proteins and DNA in this mechanism and not to their abrupt rise as required by experiment. This may be easily shown by setting all lag times equal to zero. On the other hand, the replacement of a lag time by a linear chain of ordinary reaction steps will also produce a steep rise in products [8] where, however, the actual number of reaction steps and their individual rate constants are unknown. Furthermore, multiple reaction steps would make it difficult to achieve a direct correlation between product rise times and the location of the genes as done in this work.

One of the oversimplifications of the present calculations concerns the absolute species concentrations which are not known experimentally. In fact, all rate constants used represent reduced values arising from a computer fit with the reduced concentrations obtained from experiment. Thus, the number of single protein molecules in a given bacterium is still unknown although general concentration ranges may be estimated.

Our main concern in this first attempt of a computer simulation of phage infection is to point out the significance of delay times in the T3/T7 bacteriophage systems. This work shows how delay times may be treated in a kinetic mechanism. The transcriptional lag times arise from the unique three-stage DNA transport, since we assume that transport is rate-limiting in T3/T7 transcription. On the other hand, translational lag times may be generally used in the simulation of translation processes which occur by linear diffusion of ribosomes along the messenger RNA. Delay times might be generally usable in translation as well as transcription processes of linear polymers where a given process completely excludes another process during a certain time interval. In general, linear diffusion of a protein along a linear macromolecule may give the basis for the formulation of a delay time.

When time coherence has been lost in a steady state, delay times become kinetically unimportant, since the distinct delay times have been averaged out over the total reaction time. Thus, delay times show their kinetic effects at the beginning of a coherent transient process. It will be noted that our simulations are made on the basis of a thermodynamically closed system, since all substrates are depleted inside the bacterium at the end of the infection cycle. On the other hand, the kinetics of evolution may be investigated only in an open system, where competition between phage mutant species may lead to interesting selection behavior [23].

The present calculations may be extended to include eventually all other protein species and to provide ultimately a quantitative understanding of all important processes in T3/T7 phage infection.

Our calculations have shown that it has not been possible to distinguish between an autocatalytic and a simple template mechanism for T3/T7 phage infection due to the fact that host DNA hydrolysis seems to be rate-determining. Nevertheless, the use of lag times offers some illuminating aspects of phage infection. A perhaps humorous remark may be allowed that instead of learning about life, we learned about the use of lag times in this first attempt of the simulation of T3/T7 bacteriophage infection of *E. coli* bacteria.

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References

1 M. Eigen and P. Schuster, Naturwissenschaften 64 (1977) 541.
2 M. Eigen and P. Schuster, Naturwissenschaften 65 (1978) 7.

3 M. Eigen and P. Schuster, Naturwissenschaften 65 (1978) 34.
4 J.J. Dunn and F.W. Studier, J. Mol. Biol. 166 (1983) 477; F.W. Studier and J.J. Dunn, Cold Spring Harbor Symp. Quant. Biol. 47 (1983) 999.
5 S.K. Zavriev and M.F. Shemyakin, Nucleic Acids Res. 10 (1982) 1635.
6 R. Hausmann and E. Härle, Proc. Eur. Biophys. Congr. 1, eds. E. Broda, A. Locker and H. Springer-Lederer, 1 (1971) 467.
7 N.G. Nossal, Annu. Rev. Biochem. 53 (1983) 581.
8 N. McDonald: Time lags in biological models, lecture notes in biomathematics, ed. S. Levin (Springer-Verlag, Berlin, 1978) p. 1.
9 F.W. Studier, Science 176 (1972) 367.
10 D.H. Krüger and C. Schroeder, Microbiol. Rev. 45 (1981) 9.
11 C.W. Fuller and C.C. Richardson, J. Biol. Chem. 260 (1985) 3185.
12 C.W. Fuller and C.C. Richardson, J. Biol. Chem. 260 (1983) 3197.
13 W.T. McAllister and C. Morris, J. Mol. Biol. 153 (1981) 527.
14 M.S. Center, F.W. Studier and C.C. Richardson, Proc. Natl. Acad. Sci. U.S.A. 65 (1970) 242.
15 C. Kerr and P.D. Sadowski, J. Biol. Chem. 247 (1972) 311.
16 R.L. Lechner and C.C. Richardson, J. Biol. Chem. 258 (1983) 11185.
17 T.J. Kelly and C.A. Thomas, J. Mol. Biol. 44 (1969) 459.
18 R.A. Schlegel and C.A. Thomas, J. Mol. Biol. 68 (1972) 319.
19 J.D. Watson, Nat. New Biol. 239 (1972) 197.
20 R.L. Lechner, M.J. Engler and C.C. Richardson, J. Biol. Chem. 258 (1983) 11174.
21 D. Dressler, J. Wolfson and M. Magazin, Proc. Natl. Acad. Sci. U.S.A. 69 (1972) 998.
22 S.W. Matson and C.C. Richardson, J. Biol. Chem. 260 (1985) 2281.
23 F. Buchholtz and F.W. Schneider, Ber. Bunsenges. Phys. Chem. 89 (1985) 165; Ber. Bunsenges. Phys. Chem. 89 (1985) 685.