Intermediates in the Synthesis of Phage P22 DNA

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The temperate phage P22 can grow in a lytic cycle on *Salmonella typhimurium*, or it can lysogenize this host. The DNA found in phage particles is a single linear duplex molecule (26 to 27 million mol wt) which is terminally repetitious and circularly permuted in its nucleotide sequence (Rhoades et al., 1968). As expected from these properties of the DNA, the genetic map (in vegetative crosses) has been shown to be circular (Gough and Levine, 1968). However, when the phage genome is inserted into the bacterial chromosome of the host, the phage genetic markers are arranged in a unique linear sequence (Smith and Levine, 1965; Smith, 1968).

Some of the properties of the P22-*Salmonella* system can be interpreted using models of DNA replication and lysogenization originally proposed to account for similar properties in other bacteriophages.

The Campbell model for insertion of the prophage into the host chromosome (Campbell, 1962; Signer, 1968), originally applied to the coliphage λ, may apply as well to phage P22. In fact, a major prediction of this model, that circular DNA molecules should be found in cells destined for lysogeny, has been confirmed for P22 by Rhoades and Thomas (1968). In P22 the formation of circular molecules is necessarily more complex than in λ because of the difference in the mature forms of the phage DNA. In the case of λ only ligation (a function of which the host is capable) is required (Bode and Kaiser, 1965; Gellert, 1967), whereas in the case of P22 either a recombination event or the stripping of about 1000 nucleotides from either end of the linear molecule, followed by annealing and ligation is required (Rhoades and Thomas, 1968). The formation of substantial numbers of circular duplex molecules in P22-infected cells destined for lysogeny thus has added significance, since circle formation is not an automatic consequence of the action of pre-existing host enzymes and, as shown below, rarely occurs in infections not leading to lysogeny.

Another model, originally proposed by Streisinger and his collaborators (Streisinger et al., 1964; Sechaud et al., 1965; Streisinger et al., 1967) to explain terminal repetition and circular permutation in the DNA of coliphage T4, may also apply to phage P22. The Streisinger model envisions the formation, during phage reproduction, of DNA molecules longer than those found in phage particles. These long molecules are cut into 'headfuls' which are encapsulated. If the long molecules consist of phage genomes serially repeated, and if the 'headful' contains somewhat more DNA than is required to encode the complete set of phage genes, then this scheme elegantly accounts for terminal repetition, circular permutation, and the genetic consequence of these features in the DNA: the circular vegetative map. Long molecules of the kind predicted by this model are produced during lytic cycles of P22 phage growth (Botstein, 1968; Botstein and Levine, 1968); they appear as a metabolic intermediate in DNA maturation.

Some of the topological transformations of the phage DNA during the phage life cycle are outlined in Fig. 1. During infections leading to lysogeny, substantial numbers of closed circular duplex molecules can be found, in which the terminal repetition is presumably absent. During lytic infections and after induction of lysogens, molecules 2–5 times the length of the mature phage DNA are formed; these presumably consist of several genomes laid end to end. During maturation and encapsulation these molecules are cut to the mature phage DNA length. In Fig. 1 we have included only those intracellular DNA forms whose structure is reasonably well established and which appear in substantial amount. Additional forms appearing in small quantities are not excluded; for example, small numbers of circular molecules might be formed after induction of lysogens and might even be obligatory intermediate forms.

The topological transformations shown in Fig. 1 describe what happens to the intracellular phage DNA after replication. A major intracellular DNA intermediate involved in replication is omitted—namely, the form in which active DNA synthesis takes place, regardless of the topological fate of the DNA. This intermediate (called intermediate I [Botstein, 1968]) appears to be a complex of replicating phage DNA with other cell

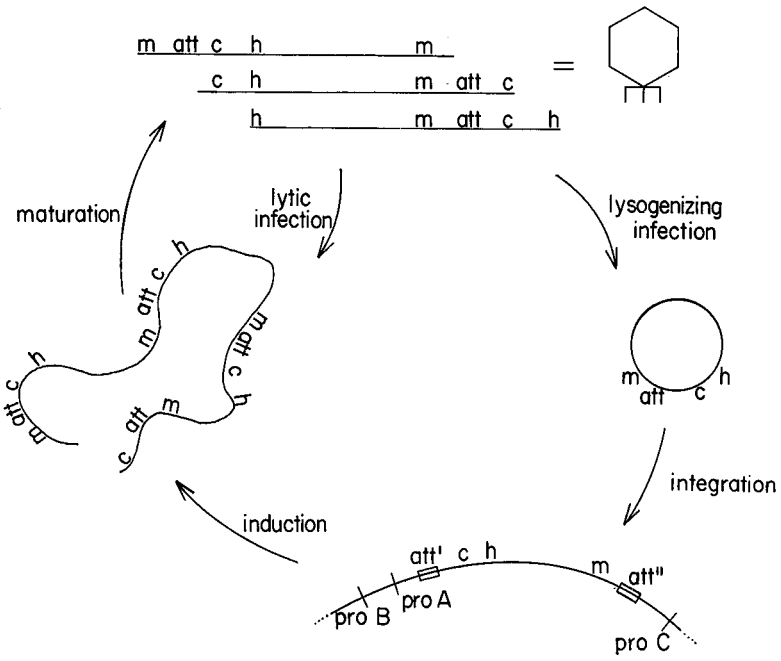

FIGURE 1. Topological rearrangements in the phage DNA observed during the phage life cycle. *m*, *c*, and *h* are morphological genetic markers on the phage linkage map. *pro*A, *pro*B and *pro*C are loci on the genetic map of the host.

constituents, possibly including the cell membrane. This replication complex is the primary subject of the remainder of this paper.

EXPERIMENTAL METHOD

The basic experimental approach is to extract replicating DNA from infected or induced cells as gently as possible. The technique was adapted from the one described by Frankel (1966) and employs lysozyme, detergent at 65°C, and extreme care in handling the lysates. Lysates are analyzed primarily by sucrose density-gradient centrifugation at neutral or alkaline pH. The gradients have cushions of high density material at the bottom to facilitate recovery of rapidly sedimenting materials (S. Altman and L. S. Lerman, in prep.). By using such gentle techniques one can obtain bacterial DNA with very high sedimentation rates. This is illustrated in Fig. 2, which shows the profile obtained when the uniformly labeled DNA of uninfected cells is sedimented through a neutral sucrose gradient with phage DNA and whole, unlysed phage particles. On the basis of the empirical equations of Studier (1965) and the known sedimentation rate of the phage particles, we estimate the sedimentation rate of the bacterial DNA to be about 250 S. This rate is consistent with the supposition that the entire bacterial genome was extracted intact.

The experiments are all of essentially the same design: cells actively synthesizing phage DNA are prepared, often by infection with ^{32}P-labeled phage, and pulses of ^{3}H-thymidine are administered. Pulses are sometimes followed by a chase with unlabeled thymidine. The cells are lysed and the DNA examined as described above. The details are to be found in Botstein (1968).

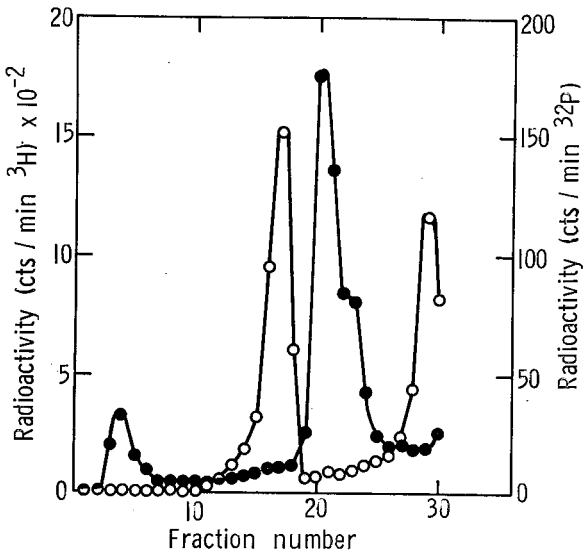

FIGURE 2. Sedimentation rate of bacterial DNA. Cells labeled uniformly with ^{3}H-thymidine were lysed together with a small number of ^{32}P-labeled phage. This lysate was layered on a 5–20% sucrose gradient (pH 8) with whole, unlysed, ^{32}P-labeled phage particles. Thus the gradient contains two ^{32}P-labeled species: phage DNA and intact phage particles. The gradient was centrifuged for 30 min at 25,000 rpm at 23°C. (– – ○ – – ○ – –): ^{32}P radioactivity. (– – ● – – ● – –): ^{3}H radioactivity. Sedimentation is from right to left in this and all subsequent figures.

TABLE 1. DISTRIBUTION OF RADIOACTIVITY INCORPORATED DURING A 1-MIN PULSE OF ^{3}H-THYMIDINE

Time after infection or induction (min)	Lytic infection		Lysogenizing infection		Temperature induction	
	Radioactivity incorporated (count/min)	Recovery in intermediate I (%)	Radioactivity incorporated (count/min)	Recovery in intermediate I (%)	Radioactivity incorporated (count/min)	Recovery in intermediate I (%)
5	727	56	562	63	—	—
10	564	59	330	65	462	61
20	4422	59	—	—	1392	56
35	8130	56	—	—	1536	60

Lytic infection: 20 c_1 phage/cell
Lysogenizing infection: 20 c^+ phage/cell
Temperature induction: shift of strain 18(tsc_2) to 39°C
Intermediate I: count/min in the first six fractions of a neutral sucrose gradient containing a high-density cushion at the bottom. Conditions of centrifugation are the same as those in the legend to Fig. 2. In these gradients intermediate I is the only identifiable component. The remainder of the DNA is spread evenly through the gradient. Recentrifugation of isolated intermediate I reproduces the pattern: about 60% of the material at the bottom of the gradient and the rest spread across the rest of the gradient.

INTERMEDIATE I: THE REPLICATION COMPLEX

A 'replicating form' of phage DNA distinguishable from nonreplicating intracellular DNA should be preferentially labeled during a short pulse of ^{3}H-thymidine. Cells actively synthesizing phage DNA were labeled for one minute, lysed immediately, and the lysates centrifuged through neutral sucrose density gradients under the same conditions used for Fig. 2. Three situations were examined: lytic infection, infection leading to lysogeny, and induction of a lysogen. In all cases (Table 1), when phage DNA synthesis is occurring, most of the incorporated radioactivity is recovered at the bottom of the gradients, in a position indicating a sedimentation rate of at least 1000 S. Tangling with or trapping by bacterial DNA cannot account for this result, since host DNA sediments much more slowly (Fig. 2). The fast-sedimenting fraction is designated as intermediate I, and appears to be the 'replicating form' or complex, possibly modified by our extraction procedure. Although intermediate I appears to be the site of phage DNA synthesis under all conditions, it has been studied primarily in lytic infections.

THE FATE OF PULSE-LABELED AND PARENTAL DNA DURING LYTIC INFECTION

The results of a typical pulse-chase experiment are shown in Fig. 3 and 4. Cells were infected with ^{32}P-labeled c_1 phage (which invariably cause lytic infection) under conditions in which about $\frac{2}{3}$ of the parental DNA fails to participate in the infection and thus serves as a marker for the mature phage position. A two-minute pulse of ^{3}H-thymidine was administered at 8 min after infection and chased with excess unlabeled thymidine. In Fig. 3 we can see most of the pulse-labeled DNA and some parental DNA in intermediate I (on the cushion of high-density material at the bottom of the gradients) early in infection (frame 1); later, intermediate II (sedimenting 1.3–1.7 times as rapidly as mature phage DNA) becomes more prominent (frames 2 through 5); and finally (at the end of the latent period) most of the radioactivity cosediments with the marker (frame 6). In alkali (Fig. 4), at early times much of the DNA in intermediate I (frame 1) is seen to sediment at about the same rate as mature phage DNA; when intermediate II is prominent in the neutral gradients (frames 2–4), a corresponding material is seen sedimenting 1.2–1.5 times as rapidly as the mature DNA. The pulse-labeled DNA cosediments with the marker at the end of the latent period in alkaline as well as in neutral gradients (frame 6).

The pulse-labeled DNA in this experiment was identified as being phage-specific by DNA-DNA hybridization. About 70% of the pulse-labeled DNA and about 30% of the parental DNA was recovered in the progeny phage.

Intermediate I thus has several properties expected of the 'replicating form.' It is preferentially labeled in a short pulse, it contains parental DNA, and the DNA in it is a precursor of the DNA in the progeny.

THE NATURE OF INTERMEDIATE I IN LYTIC INFECTIONS

It is probable that intermediate I represents a complex of DNA with other cell materials and that the DNA in this complex is primarily in the classical double-helical conformation. The basis for this belief follows.

1. The sedimentation rate characteristic of intermediate I (at least 1000 S) is attained by the DNA of a temperature-sensitive phage mutant (ts12.1) which does not synthesize measurable

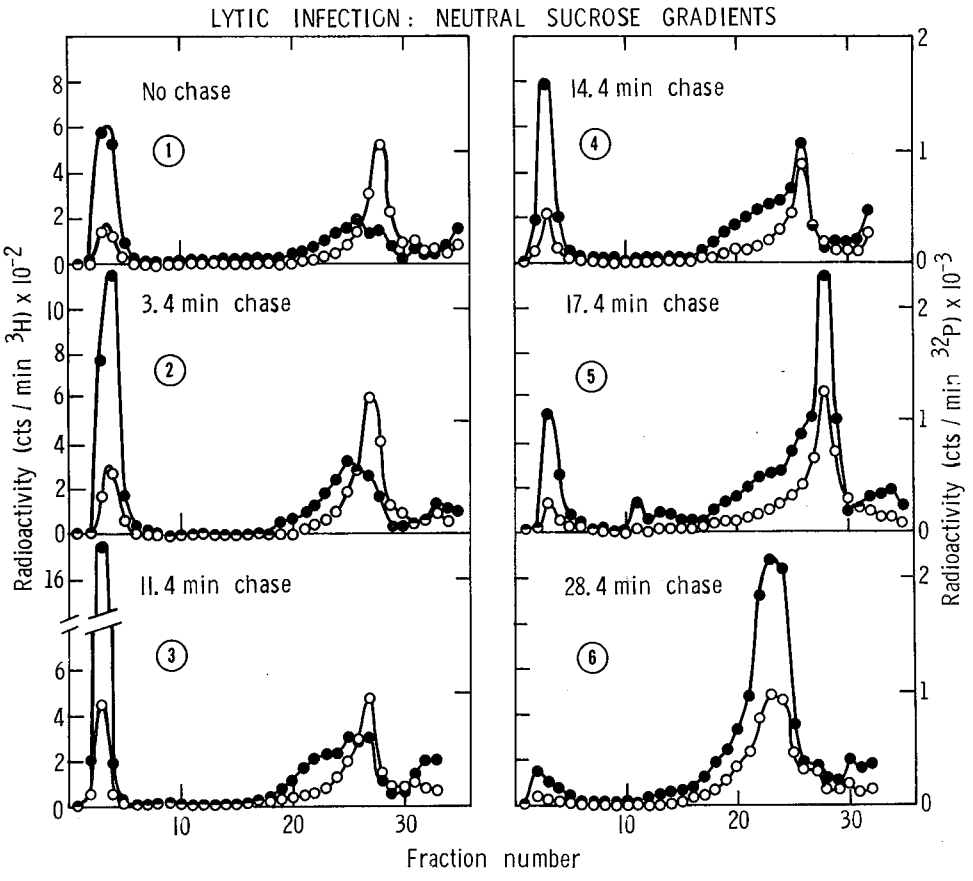

FIGURE 3. The fate of parental and pulse-labeled phage DNA during lytic infection: neutral sucrose gradients. Cells were infected with 20 c_1 phage/cell. ^{3}H-thymidine was added at 8 min after infection and excess unlabeled thymidine was added at 10 min after infection. Lysates (made at the indicated times) were centrifuged through neutral 5–20% sucrose gradients which had a cushion of high-density material at the bottom for 60 min at 35,000 rpm at 23°C. Recovery of radioactivity was always better than 87% (– – ○ – – ○ – –): ^{32}P radioactivity. (– – ● – – ● – –): ^{3}H radioactivity.

TABLE 2. THE RELATIVE SENSITIVITY OF ^{3}H-LABELED INTERMEDIATE I DNA AND ^{32}P-LABELED MATURE PHAGE DNA TO DNases OF DIFFERENT SPECIFICITIES

Form of DNA substrates	Enzyme	Radioactivity rendered acid soluble (count/min)* ^{32}P	^{3}H	Extent of digestion (per cent) ^{32}P	^{3}H
Both native	exo I	0	6	0.2	0.9
	Neurospora endo	0	3	0.2	0.5
	λ exo (3)†	58	80	11.	12.
	pancreatic	178	289	36.	44.
^{32}P denatured and ^{3}H native	exo I	510	8	100.	1.2
	Neurospora endo	182	5	36.	0.8
	λ exo (3)†	21	89	4.	14.
Both denatured	exo I	502	582	98.	89.
	Neurospora endo	213	253	43.	39.
	λ exo (30)†	42	70	8.	11.
	pancreatic	95	132	19.	20.

Each of the incubation mixtures contained 1021 count/min ^{32}P-labeled purified phage DNA and 1312 count/min ^{3}H-labeled isolated intermediate I DNA. Since half of the incubation mixture was assayed for acid soluble radioactivity at the end of the incubation, the total possible recoverable radioactivity was 511 count/min ^{32}P and 656 count/min ^{3}H. Denaturation of DNA was accomplished by heating to 95°C for 15 min followed by rapid cooling in an ice-water bath.

* Blank values are subtracted; they were always less than 5 count/min.

† The number in parenthesis indicates the number of units of λ exonuclease used.

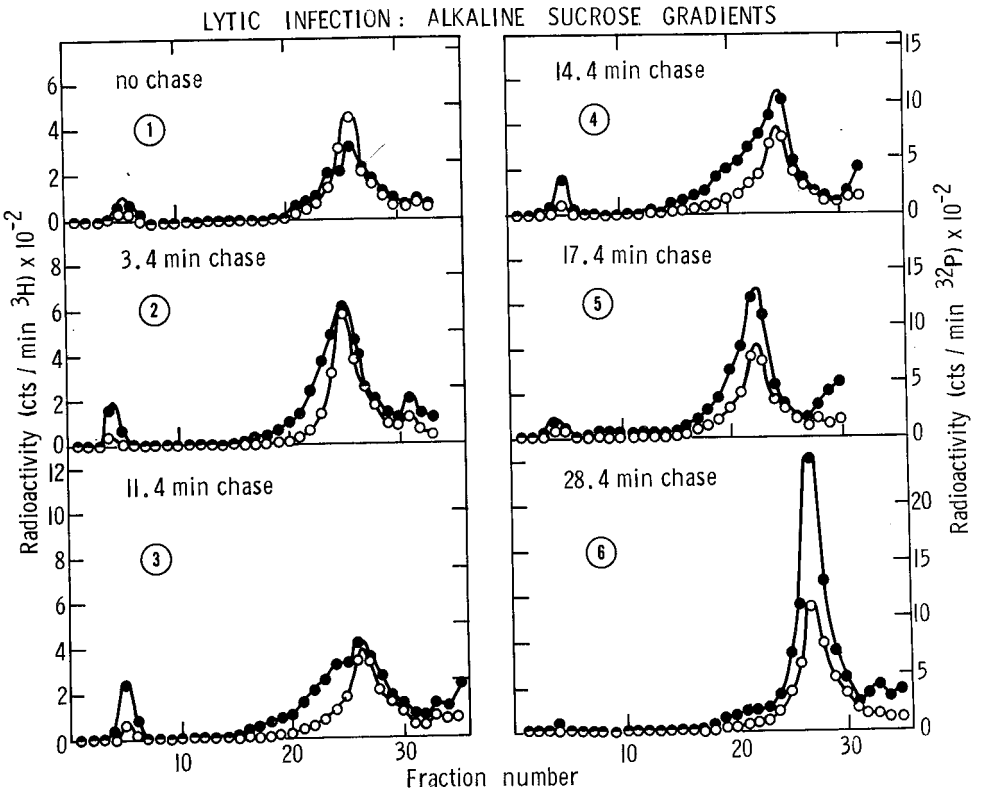

FIGURE 4. The fate of parental and pulse-labeled phage DNA during lytic infection: alkaline sucrose gradients. The origin of the lysates is described in the legend to Fig. 3. The pH of the gradients was 12.1. The lysates were brought to alkaline pH on top of the gradients by layering the appropriate amount of NaOH before layering the lysates. Centrifugation was at 35,000 rpm for 90 min at 23°C. Recovery of radioactivity was always better than 79%. (– – ○ – – ○ – –): ^{32}P radioactivity. (– – ● – – ● – –): ^{3}H radioactivity.

amounts of DNA at restricting temperature (Botstein and Levine, 1968). No conformational change in DNA is known which would raise the sedimentation rate from 33 S to 1000 S. Even in the denatured collapsed form, pure DNA would have to be several hundred times the mature phage size to sediment so rapidly (cf. Studier, 1965).

2. The DNA in intermediate I has a susceptibility to nucleases of known specificity which is not very different from that of authentic mature phage DNA. Table 2 shows the results obtained when mixtures of ^{32}P-labeled mature phage DNA and ^{3}H-labeled intermediate I DNA (from a lytic infection) were incubated with the nucleases. Three mixtures were made: one contained both kinds of labeled DNA mixed together without further treatment, another contained untreated intermediate I DNA and heat-denatured mature phage DNA, and the third mixture contained both kinds of DNA in the heat-denatured form. Each mixture was incubated with exonuclease I (Lehman, 1960), which is specific for single-stranded DNA with free 3′-hydroxyl ends (Lehman and Nussbaum, 1964); with the *Neurospora crassa* endonuclease, which will attack DNA only if it has regions of disordered structure (Linn and Lehman, 1965a and b); with the λ exonuclease (Korn and Weissbach, 1963; Radding, 1966) which preferentially attacks double-stranded DNA with free 5′-phosphoryl ends (Little, 1967); and finally with pancreatic DNase, which will digest DNA regardless of secondary structure. Except for the possibility that denatured intermediate I DNA may be partially resistant to exonuclease I, no difference in susceptibility to any of these enzymes was detected between intermediate I DNA and mature DNA. Particularly interesting is the failure to find, under these conditions, any evidence for single-stranded regions in intermediate I DNA.

3. Intermediate I is partially stable to alkali. At pH 12.1, about one third of the labeled DNA still sediments with a coefficient of at least 800 S. The remainder of the DNA sediments at or near the mature DNA rate, depending on the conditions of infection and the phenotype of the infecting phage (Botstein, 1968; Botstein and Levine, 1968). Since DNA is in an extended form without secondary structure at this pH (Studier, 1965), the

assumption that intermediate I consists of DNA alone would give some of that DNA a molecular weight which is unreasonable by orders of magnitude.

The Formation of Intermediate I

Since parental phage DNA appears in intermediate I, we can observe the formation of the complex by infecting cells with ^{32}P-labeled phage. Using this system, we have been able to learn the following:

1. Substantial DNA synthesis is not required for the formation of intermediate I, since the DNA of a temperature-sensitive mutant (*ts*12.1) which fails to synthesize measurable quantities of phage DNA, nevertheless appears in the complex (Botstein and Levine, 1968).

2. Intermediate I formation is correlated with successful infection. In strains of Salmonella which plate phage P22 with an efficiency of about 0.3 (Bezdek and Amati, 1967), only about 25% of the parental phage DNA appears in the complex. In strains which plate the phage with an efficiency near 1, essentially all the parental DNA can be recovered in intermediate I.

3. There are many sites for binding of parental DNA into the replication complex. The proportion of parental phage DNA in intermediate I is invariant with multiplicity of infection in the range 0.5–50 phage/cell (Botstein and Levine, 1968).

4. The formation of intermediate I is under immunity control. Lysogenic cells (strain 18 [$c^+h^+sie_1$]) which do not exclude superinfecting phage but which are immune (Rao, 1968; Walsh and Meynell, 1967) were superinfected with ^{32}P-labeled c_1h_{21} phage. Nonlysogenic strain 18 cells were similarly infected as a control. At various times after infection each culture was lysed after having been given a 2-min pulse of ^{3}H-thymidine and the DNA sedimented through a neutral sucrose gradient. Figure 5a shows the result in the control infection at 12 min after infection. The normal situation is observed—essentially all the pulse-labeled DNA and about 25% of the parental DNA

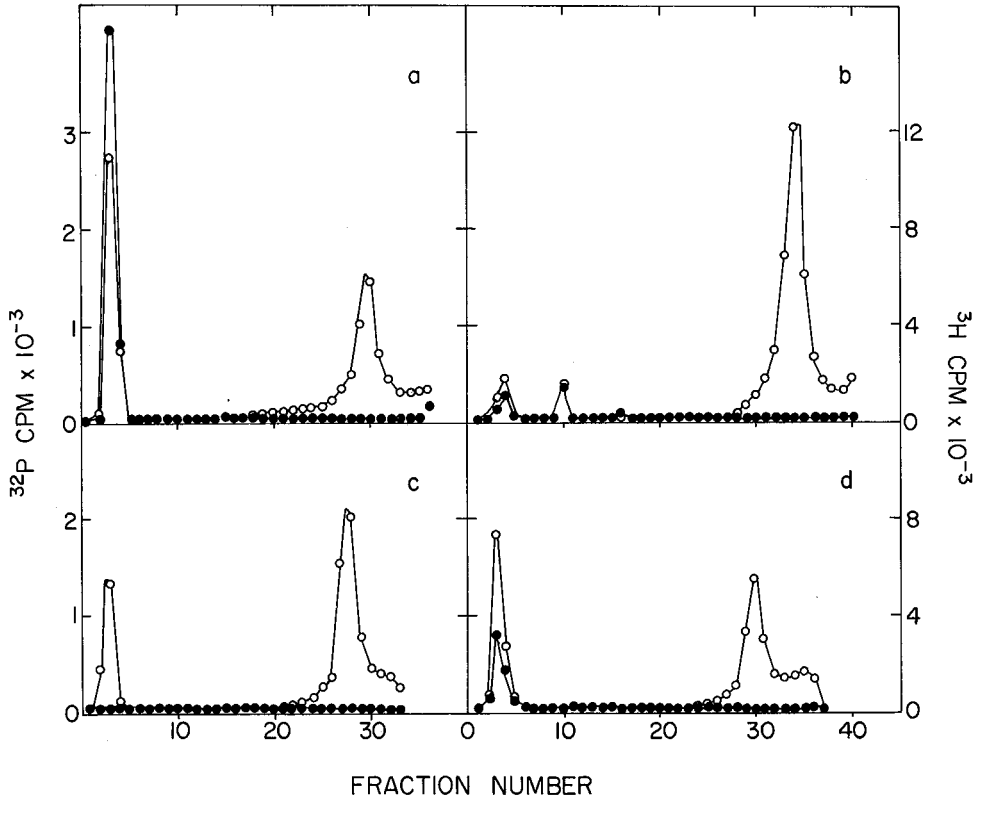

Figure 5. Formation of the replication complex during homo-immune superinfection. The plan of the experiment is described in the text. Lysates were centrifuged through neutral sucrose gradients as described in the legend to Fig. 3. (a) Infection of sensitive cells with ^{32}P-labeled phage. ^{3}H-thymidine added at 10 min after infection, lysate made at 12 min after infection. (b) Homo-immune superinfection with ^{32}P-labeled phage. ^{3}H-thymidine added at 10 min after infection, lysate made at 12 min after infection. (c) Homo-immune superinfection with ^{32}P-labeled phage followed by UV irradiation at 14 min after infection. ^{3}H-thymidine added at 30 min after infection, lysate made at 32 min after infection. (d) Same as (c), except that ^{3}H-thymidine was added at 50 min after infection and the lysate made at 52 min after infection. (– – ○ – – ○ – –): ^{32}P radioactivity. (– – ● – – ● – –): ^{3}H radioactivity.

is recovered at the bottom of the gradient, in intermediate I. However, in the superinfection (Fig. 5b) very little ^{3}H-thymidine was incorporated and correspondingly little parental DNA is in the intermediate I position. The small amount observed is probably due to titration of repressor by the superinfecting genomes and consequent induction in a small fraction of the cells (Rao, 1968). In order to show that the failure to form intermediate I is due to immunity, and not to trivial exclusion or adsorption phenomena, a superinfected lysogen was given an inducing dose of ultraviolet light at 14 min after superinfection. In this case, at 32 min after infection (Fig. 5c) parental DNA appears in intermediate I, but there is little incorporation of ^{3}H-thymidine. At 52 min, (Fig. 5d) normal DNA synthesis is taking place, as usual, exclusively in intermediate I. Eighty per cent of these cells yielded good bursts of phage. The ratio of superinfecting to prophage types in the progeny was about 1.5.

5. Entry of parental DNA into the complex probably precedes its replication. This assertion is based on the behavior of the DNA negative strain (*ts*12.1) and on the results displayed in Fig. 5c and 5d.

Removal of DNA from the Replication Complex During DNA Maturation

As shown in Fig. 3, intermediate I persists for a while during the chase and then disappears as intermediate II and mature phage DNA are formed. Figure 6 clearly shows the simultaneous disappearance of parental and newly synthesized DNA from intermediate I, beginning at 20 min after c_1 infection. Also shown is a similar experiment carried out with a temperature-sensitive phage mutant (*ts*5.1) which suggests that it is defective in the removal of DNA from intermediate I.

DNA begins to disappear from intermediate I at 20 min after c_1 infection, at the time the late phage functions are turned on. The *ts*5.1 mutation has been shown by temperature-shift experiments to affect a late function (Levine, unpubl. results). Under conditions which result in failure to turn on late functions (infection with an early mutant [*ts*12.1] or infection leading to lysogeny) the complex is stable for more than two hours. Thus we conclude that the removal of DNA from the replication complex is a late phage function.

The Production of Phage DNA Strands Longer than Those of Mature Phage DNA During Lytic Infections

Intermediate II (see the discussion of Fig. 3 and 4, above) was isolated from a neutral gradient,

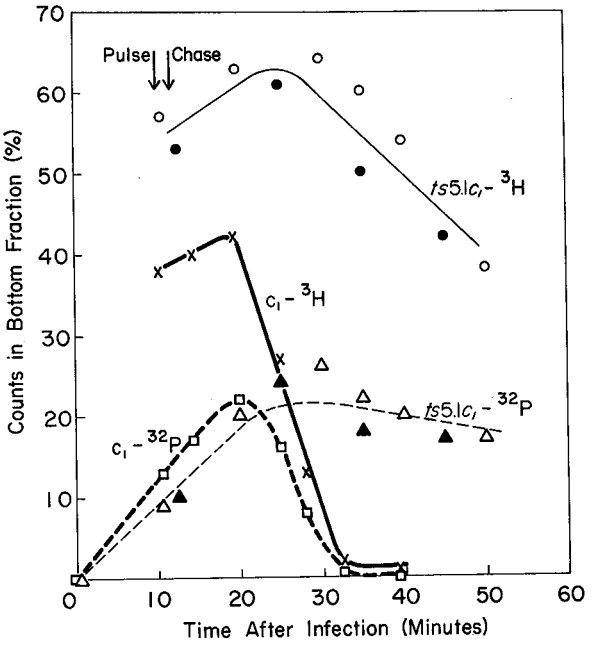

FIGURE 6. The proportions of parental and pulse-labeled phage DNA in intermediate I at various times during lytic infection. The data are derived from the gradients of Fig. 3 and from a similar experiment carried out with ^{32}P-labeled *ts*5.1c_1 phage at 39°C (the restrictive temperature). Two separate experiments with different preparations of *ts*5.1c_1 phage are shown. The fraction of total radioactivity in intermediate I was calculated by dividing the sum of the radioactivity in the first six fractions of each sucrose gradient by the total radioactivity in the gradient. (– – ○ – – ○ – –) and (– – ● – – ● – –): pulse-labeled DNA (^{3}H) in intermediate I during infection with *ts*5.1c_1 phage. (– – △ – – △ – –) and (– – ▲ – – ▲ – –): parental DNA (^{32}P) in intermediate I during infection with *ts*5.1c_1 phage. (—X—X—): pulse-labeled DNA (^{3}H) in intermediate I during infection with c_1 phage. (– – □ – – □ – –): parental (^{32}P) DNA in intermediate I during infection with c_1 phage.

treated extensively with pronase, dialyzed, and centrifuged through neutral and alkaline sucrose gradients with purified differentially labeled mature phage DNA. The results (Fig. 7) show that intermediate II sediments more rapidly than mature phage DNA in both neutral and alkaline sucrose gradients. The relative sedimentation rates are consistent with the hypothesis that the intermediate II DNA strands are longer than those of the mature phage DNA. The density of intermediate II in neutral CsCl, alkaline CsCl, and CsCl-ethidium bromide density gradients (Radloff et al., 1967) indicates that intermediate II consists entirely of linear double-helical DNA. This DNA was identified as phage-specific by hybridization (Botstein, 1968).

DNA strands longer than the mature phage DNA length also appear in intermediate I under certain circumstances. After infection with strain *ts*5.1c_1, which fails to release DNA from

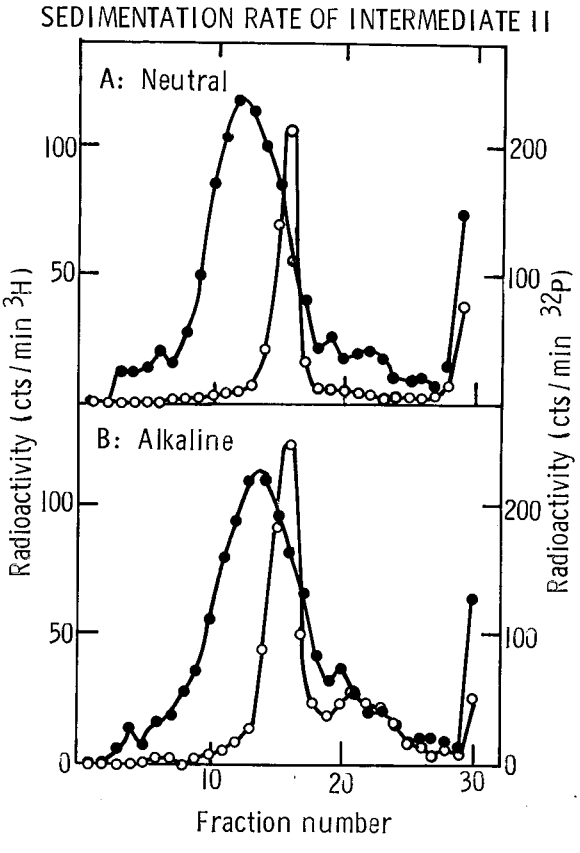

FIGURE 7. Sedimentation rate of isolated intermediate II. Cells infected with 20 c_1 phage/cell were labeled with ^{3}H-thymidine ten min after infection. At 28 min after infection the cells were lysed and the lysate centrifuged through a 30 ml neutral sucrose gradient. The fractions in the intermediate II region were pooled, treated overnight with 1 mg/ml pronase, and dialyzed for 2 days (with more pronase) against 0.01 M Tris, pH 7.5. The isolated material was mixed with purified, ^{32}P-labeled phage DNA and applied to the following 5 ml gradients: (A) 5–20% sucrose gradient containing 1.0 M NaCl. 0.02 M Tris, and 0.01 M EDTA, pH 8. 0.1 ml of the mixture was applied. (B) 5–20% sucrose gradient containing 0.9 M NaCl, 0.1 M NaOH (the solutions were titrated to pH 12.1; actually, the concentration of NaOH is somewhat greater than 0.1 M), and 0.005 M EDTA. 0.1 ml of 0.25 M NaOH was layered on the gradient before the application of the sample (0.1 ml also). The completely layered gradient was allowed to stand for 30 min before centrifugation. Centrifugation in both (A) and (B) was for 135 min at 35,000 rpm at 23°C. In both (A) and (B) there was better than 85% recovery of all radioactivities. (– – ○ – – ○ – –): ^{32}P radioactivity (purified phage DNA marker); (– – ● – – ● – –): ^{3}H radioactivity (isolated intermediate II).

intermediate I, strands up to ten times the mature length have been detected by sedimentation through alkaline sucrose gradients (Botstein and Levine, 1968).

Little, if any, of the DNA in intermediate I is in the closed circular duplex conformation during lytic infections. We failed to detect any closed circular duplex molecules using CsCl-ethidium bromide equilibrium density gradients.

PHAGE GENES IMPLICATED IN DNA MATURATION DURING LYTIC INFECTIONS

Mutations in gene 5 cause failure to release DNA from intermediate I. Mutations in gene 15 produce a similar phenotype. Strains with mutations in gene 10 are normal with respect to the formation and dissolution of intermediate I but fail nevertheless to produce mature phage DNA. These strains accumulate DNA in a form resembling intermediate II. Mutations in two other genes (8 and 13) result in incomplete or abnormal DNA maturation.

All five genes thus far implicated in DNA maturation appear to code for late functions and they form a cluster on the genetic map (M. Gough, unpubl. results).

DISCUSSION

Phage DNA synthesis appears always to take place in the replication complex (intermediate I) even though the topological fate of intracellular phage DNA depends upon the conditions of phage growth. The role of intermediate I has been studied in some detail only in lytic infections, and the conclusions may be summarized as follows:

All parental DNA molecules competent to initiate infection are attached to some other cell material (possibly the cell membrane) to form the replication complex (intermediate I). At least 50 molecules may be bound in a single cell. Although the binding does not seem to require DNA synthesis, formation of the complex is at least in part a phage function under the control of the immunity system. Binding of parental DNA probably precedes replication and appears to be a necessary but not a sufficient condition for phage DNA synthesis. Removal of DNA from the complex appears to be a late phage function.

The DNA in the replication complex is primarily in the ordinary double-stranded conformation. During lytic infections little, if any, of this DNA is in the closed circular conformation described by Rhoades and Thomas (1968) and found during infections leading to lysogeny. It is possible, however, that some intermediate I DNA is circular in only one strand during lytic infection. Under certain conditions much of the DNA in intermediate I consists of chains substantially longer than those of the mature phage DNA.

The origin of the long strands of DNA found in intermediate II is uncertain. That long strands are found in intermediate I under some conditions suggests that strand elongation may normally occur while the DNA is in the replication complex. Strand elongation may be the result of replication (for example, according to the rolling circle

model [Gilbert and Dressler, this volume]) or the result of recombinations between progeny molecules, either at the terminal redundancy, or after permutation of their sequence.

Of particular interest at the present time are the mechanisms which determine whether the DNA synthesized in the complex is converted into circular forms (during lysogenization) or into long strands (during lytic infection). A mutation in the c_1 gene results in lytic infection under conditions that normally produce lysogeny. (In P22 the c_1 gene is *not* the immunity repressor; the c_2 gene appears to be analogous to the c_I of λ.) Smith and Levine (1964) showed that the c_1 gene product is responsible for the first of a series of functions repressing phage DNA synthesis during lysogenization. Rhoades and Thomas (1968) found that circle formation occurs only after the c_1 repression has occurred. Botstein (1968) found that long forms appear late in lytic infections. It appears that inhibition of DNA synthesis resulting from the action of the c_1 gene product is directly involved in the topological fate of the phage DNA in the replication complex.

Acknowledgments

We thank M. S. Fox and E. R. Signer for their helpful criticism of the manuscript. The work was supported by grants from the National Institutes of Health: No. A1-05388-06 to M. S. Fox at the Massachusetts Institute of Technology and No. GM-09525-05 to the Department of Human Genetics, University of Michigan.

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