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Synthesis and Maturation of Phage P22 DNA

II. Properties of Temperature-sensitive Phage Mutants Defective in DNA Metabolism

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Three temperature-sensitive mutants of phage P22 have been isolated which are defective in phage DNA metabolism. After infection at restrictive temperature, one mutant (*ts* 12.1) fails to synthesize measurable quantities of phage DNA; nevertheless, parental phage DNA is recovered in a fast-sedimenting form similar to the first intermediate form observed after infection with normal phage. Another mutant (*ts* 5.1) synthesizes normal amounts of phage DNA, but neither this DNA nor parental DNA is converted from the first, fast-sedimenting intermediate form into the second, more slowly sedimenting intermediate observed in normal infections. The third mutant (*ts* 10.1), produces both intermediate forms in sequence, but fails to produce DNA having the sedimentation properties of mature phage DNA. None of the mutants encapsulates any DNA.

The properties of these mutants are interpreted as supporting a scheme of sequential intermediates in P22 phage DNA synthesis and maturation.

1. Introduction

Two forms of intracellular phage DNA which differ in sedimentation velocity from mature phage DNA have been extracted during lytic growth cycles of phage P22 (Botstein, 1968). One of these forms, intermediate I, has a sedimentation rate exceeding 1000 S in neutral sucrose gradients. It contains most of the radioactivity incorporated in a one-minute pulse of [^{3}H]thymidine if extraction of the DNA immediately follows the pulse-labeling. The other form, intermediate II, has a sedimentation 1·2 to 1·7 times that of the mature phage DNA in both neutral and alkaline sucrose gradients. It is observed when pulse-labeling is followed by the administration of unlabeled thymidine before extraction.

A scheme is put forward in the preceding communication (Botstein, 1968) in which intermediate I corresponds to a complex in the cell which is the site of phage DNA replication. The replicated DNA is released from the synthesizing complex through the action of a late phage function and this DNA becomes, upon extraction, intermediate II. The DNA in intermediate II might be the substrate for the encapsulation of phage DNA into phage heads. According to the proposed scheme, intermediate II DNA is a precursor of the mature phage DNA.

This formulation predicts the existence of at least three classes of phage mutants: one which fails to synthesize phage DNA (parental DNA may or may not appear in

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intermediate I); one which accumulates newly synthesized phage DNA in intermediate I and produces no intermediate II or mature phage DNA; and one which causes newly synthesized phage DNA to appear in intermediate I and II in sequence but which fails to make mature phage DNA. Since these mutational blocks are expected to be lethal, they can only be obtained as conditional-lethal mutations (Horowitz & Leupold, 1951; Edgar & Lielausis, 1964; Epstein *et al.*, 1963). This paper describes three temperature-sensitive phage mutants that correspond to the three classes predicted by the scheme proposed in the preceding paper (Botstein, 1968).

2. Materials and Methods

Most of the materials and methods are described in the preceding communication (Botstein, 1968). Minor modifications were sometimes made in order to adapt the methods to temperature-sensitive phages. For example, preparation of ^{32}P-labeled temperature-sensitive phage necessitated growing the phage at 25°C instead of 37°C. Only methods not previously described are included below.

(a) *Bacteria and phage*

The bacterial strain used was strain 18 of *Salmonella typhimurium*.

The mutually complementing temperature-sensitive phage mutants, *ts* 5.1, *ts* 10.1 and *ts* 12.1 were isolated after nitrosoguanidine mutagenesis of wild-type P22 (Gough & Levine, 1968). In order to reduce the probability of multiple lesions in the strains selected for study, the following crosses were performed with each of the mutants.

(1) Each mutant was crossed with a non-temperature-sensitive strain carrying 3 morphological markers: m_3, c_2 and h_{21} (Levine, 1957; Levine & Curtiss, 1961). Temperature-sensitive recombinants carrying all 3 of these morphological markers were isolated.

(2) The temperature-sensitive mc_2h isolates were crossed with wild-type P22 phage. Temperature-sensitive recombinants lacking all 3 of the morphological markers were isolated.

(3) In order to obtain strains which will give a lytic cycle upon infection, these isolates were crossed with c_1 phage. Temperature-sensitive c_1 recombinants were isolated; the resulting strains (*ts* 5.1 c_1, *ts* 10.1 c_1 and *ts* 12.1 c_1) were used throughout this work.

The 3 temperature-sensitive phage strains thus obtained were grown on strain 18 cells at 25°C and the lysates purified in a stepwise CsCl gradient (preceding paper).

(b) *Determination of phage functions at 39°C*

Strain 18 cells, growing exponentially at 39°C in LCG20 medium, were infected with 20 phage/bacterium. Growth was followed by measuring optical density in a Klett colorimeter. After lysis was observed (or, in the case of *ts* 12.1 c_1, which does not cause lysis after infection at 39°C, after 65 min) 2 drops of chloroform were added and the bacterial debris removed by centrifugation. The supernatant fluids were assayed for plaque-forming units at 25°C. Phage functions at 39°C were assayed as follows:

(i) *Tail parts*

5×10^8 purified, non-infective heads (Israel, Anderson & Levine, 1967) were incubated with 0·1 ml. of lysate for 2 hr at 25°C. The increase of phage titer at 37°C is a measure of the ability to produce tail parts. In Table 1, + indicates essentially complete reactivation of the viable heads, i.e. 0·1 ml. of lysate reactivates all of the 5×10^8 heads.

(ii) *Viable heads*

Tail parts (purified in sucrose density-gradients (Israel *et al.*, 1967) were added in great excess to 0·1 ml. of lysate. Any increase in phage titer at 25°C indicates the presence of viable heads. None of the mutant lysates showed any significant increase in titer.

(c) *Determination of the capacity to render DNA insensitive to DNase*

Intracellular phage DNA was labeled as follows: Strain 18 cells growing exponentially at 39°C in LCG20 medium were infected with 20 temperature-sensitive phage/cell

Simultaneously, 5 μc (10 c/m-mole) [^{3}H]thymidine/ml. was added together with 250 μg deoxyadenosine/ml. After spontaneous lysis was observed, the lysate was centrifuged to remove bacterial debris.

The supernatants were digested with 5 μg pancreatic DNase (Worthington; twice crystallized)/ml. in the presence of 0·01 M-$MgCl_2$ at 37°C for 3 hr. This procedure causes less than half the acid-insoluble radioactive material in a lysate from a non-temperature-sensitive c_1 infection to become acid soluble. In the cases of strains *ts* 5.1 c_1 and *ts* 10.1 c_1, however, it causes more than 90% of the acid-insoluble radioactive material in the lysate to become acid soluble.

3. Results

(a) *Properties of three temperature-sensitive phage mutants*

The properties of three temperature-sensitive phage strains, *ts* 12.1 c_1, *ts* 10.1 c_1 and *ts* 5.1 c_1, are compared to those of the normal c_1 strain in Table 1. The data

TABLE 1

Comparison of the properties of the temperature-sensitive phage strains with normal phage

Phage strain	Burst size (phage/cell)		Lysis time (min after infection)		*Functions at 39°C*			
	25°C	39°C	25°C	39°C	Tail parts†	Viable heads†	Phage DNA synthesis‡	DNase-resistant DNA†
c_1	470	220	85	45	+§	+§	+	+
ts 12.1 c_1	380	0·1	88	no lysis	−	−	−	not tested
ts 10.1 c_1	420	0·3	90	55	+	−	+	−
ts 5.1 c_1	480	0·4	92	52	+	−	+	−

† Determined as described in Materials and Methods.
‡ Levine & Smith, 1966, *9th Int. Congr. Microbiol.* Abstr. 470; Levine, unpublished results.
§ By assumption; not actually determined.

suggest that the *ts* 12.1 mutation affects an "early" function and that strains *ts* 5.1 c_1 and *ts* 10.1 c_1 affect "late" functions. This conclusion is based on the fact that the latter two mutants synthesize DNA, base plate parts, and cause cell lysis, whereas strain *ts* 12.1 c_1 does none of these things at restrictive temperature. These assignments have been confirmed by temperature-shift experiments to be published elsewhere (Gough, Israel, Botstein, Ewing & Levine, manuscript in preparation).

Strains *ts* 10.1 c_1 and *ts* 5.1 c_1 synthesize phage DNA, but are unable to convert the DNA into a DNase-resistant form. Thus, the *ts* 5.1 and *ts* 10.1 mutations are probably defective at or before encapsulation of DNA into phage heads but after the synthesis of phage DNA.

(b) *Formation and stability of intermediate I after infection with the temperature-sensitive phage mutants*

In experiments presented in this and the following sections, cells infected with ^{32}P-labeled phage are pulse-labeled with [^{3}H]thymidine for two minutes. The parental [^{32}P]DNA and the pulse-labeled [^{3}H]DNA are then followed during chase with unlabeled thymidine.

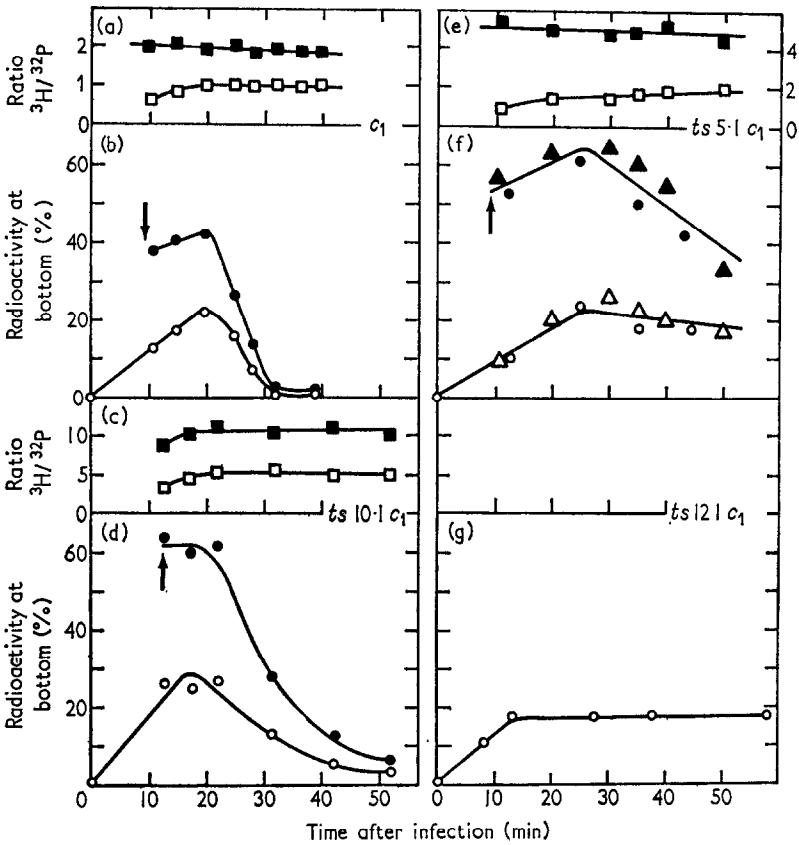

FIG. 1. Formation and stability of intermediate I after infection with the temperature-sensitive phage mutants.

(a) and (b) Infection with c_1 phage at 37°C; the data are from Fig. 7 of the preceding paper. (c) and (d) Infection with *ts* 10.1 c_1 phage at 39°C. (e) and (f) Infection with *ts* 5.1 c_1 phage at 39°C; two different experiments with different radioactive phage preparations are shown. (g) infection with *ts* 12.1 c_1 phage at 39°C.

Strain 18 cells were infected with 20 ^{32}P-labeled phage/cell at the beginning of the experiment. At the time indicated by the arrows, [^3H]thymidine (17 c/m-mole: 20 μc/ml.) was added; 2 min later unlabeled thymidine (2 mg/ml.) was added. Samples were taken, lysed and analyzed in neutral 5 to 20% sucrose density-gradients as previously described. All gradients, as usual, were made on top of a cushion of high-density material to ensure recovery of rapidly sedimenting materials. The gradients were centrifuged at 35,000 rev./min for 60 min at 23°C. Recovery of radioactivity was in all cases better than 80%. The number of fractions per gradient varied from 28 to 35.

Radioactivity at bottom is the sum of the radioactivity recovered in the first 6 fractions of each gradient.

(—△—△—) and (—○—○—) ^{32}P radioactivity (parental phage DNA); (—▲—▲—) and (—●—●—) ^3H radioactivity (pulse-labeled phage DNA). (—■—■—) Ratio of ^3H radioactivity (pulse-labeled phage DNA) to ^{32}P radioactivity (parental phage DNA) in the bottom fraction; (—□—□—) ratio of ^3H radioactivity to ^{32}P radioactivity in the unfractionated lysate.

TABLE 2

Identification of DNA pulse-labeled after infection with temperature-sensitive phage mutants by DNA–DNA hybridization

Sample source	Total acid insoluble radio-activity (cts/min)	*Radioactivity bound to filters*			Ratio of phage to bacterial binding activity†	Recovery on filters (%)‡	Proportion of phage DNA in total pulse-labeled DNA (%)§
		Blank (cts/min)	Bacterial DNA (cts/min)†	Phage DNA (cts/min)†			
ts 5.1 c_1 infection; no chase	1822	56	134	620	4·6	41	82
ts 5.1 c_1 infection; 23 min chase	2420	49	142	756	5·3	39	84
ts 10.1 c_1 infection; no chase	2252	43	110	592	5·4	31	84
ts 10.1 c_1 infection; 30 min chase	2630	47	136	685	5.0	30	83
Bacterial DNA	1421	53	496	35	0.07	37	6.5

The procedure is described in the preceding communication. The phage samples come from the experiment described in the legend to Fig. 1; the bacterial DNA from a culture of strain 18, labeled uniformly with [^{3}H]thymidine, and lysed in the usual way.

† Blank values are subtracted.

‡ The sum of radioactivities recovered on both kinds of filters compared to the total acid-insoluble radioactivity.

§ The radioactivity found on phage filters compared to the sum of radioactivities found on both kinds of filters.

Figure 1 shows that parental DNA appears in intermediate I (preceding paper), with any of the mutant strains at rates and in proportions similar to those found with c_1 phage.

With c_1 phage (Fig. 1(b)), the proportion of both parental and newly synthesized phage DNA in intermediate I decreases during the second half of the latent period. Parental phage DNA of the early mutant strain, *ts* 12·1 c_1 (Fig. 1(g)), does not show this decrease but remains extractable as intermediate I for as long as three hours. The infected cells survive, and resume normal growth within 30 minutes after infection. This strain synthesizes little phage DNA, if any (Levine & Smith, 1966, *9th Int. Congr. Microbiol.* Abstr. 470; Levine, 1967). Therefore, DNA synthesis is probably not required for entry of parental DNA into intermediate I.

With strain *ts* 5·1 c_1, as well, intermediate I is observed very late after infection (Fig. 1(f)). Phage DNA is synthesized in normal amounts, but both parental and pulse-labeled DNA disappear from intermediate I very slowly. The slow reduction in amount recovered at the bottom of the gradients might reflect asynchrony of lysis in the population, since the intermediate DNA released by cells which lyse early might be degraded by bacterial nucleases. It is nevertheless clear that, in cells infected with strain *ts* 5.1 c_1, newly synthesized DNA accumulates in the form of intermediate I.

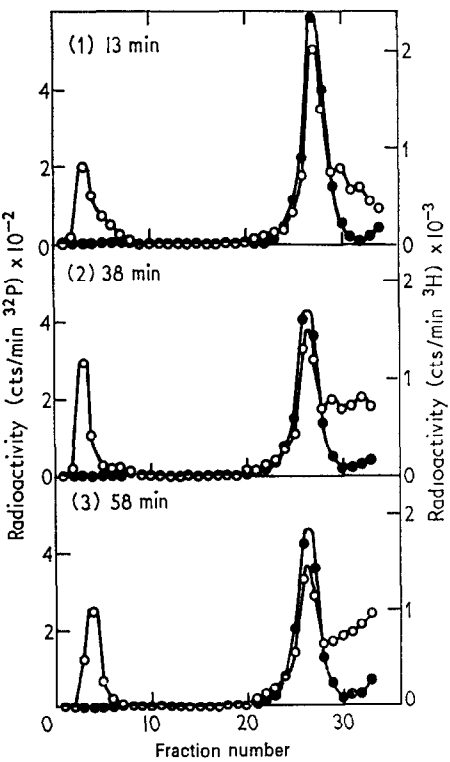

FIG. 2. Fate of parental DNA after infection with strain *ts* 12.1 c_1.

Lysates (made at the indicated times after infection) from an infection of strain 18 with 20 ^{32}P-labeled *ts* 12.1 c_1 phage/cell were centrifuged through natural sucrose gradients (preceding paper) together with purified ^{3}H-labeled phage DNA. Centrifugation was at 35,000 rev./min for 60 min at 23°C. Recoveries in all 3 cases were better than 88%. Sedimentation is from right to left in this and subsequent Figures.

(—○—○—) ^{32}P radioactivity (parental phage DNA); (—●—●—) ^{3}H radioactivity (purified phage DNA marker).

Strain *ts* 10.1 c_1 is apparently normal in DNA synthesis and maturation up to and including the removal of DNA from the *in vivo* counterpart of intermediate I (Fig. 1(d)). However, there is an increase in the time scale, probably related to the increased length of the latent period.

Figure 1 also shows the ratios of ^3H radioactivity (pulse-labeled DNA) to ^{32}P radioactivity (parental DNA) in the unfractionated lysates (lower line) and in intermediate I (upper line) (Fig. 1(a), (c) and (e)). The ratio of radioactivities in intermediate I is constant during the *latter* part of the latent period in all cases, indicating that the parental and newly synthesized phage DNA disappear from intermediate I together.

(c) *Hybridization of DNA synthesized after infection with strains* ts *5.1* c_1 *and* ts *10.1* c_1

DNA–DNA hybridization (Table 2; Botstein, 1968; Denhardt, 1966) shows that the DNA synthesized after infection with strains *ts* 5.1 c_1 and *ts* 10.1 c_1 is phage DNA. This is true for pulse-labeled DNA regardless of the length of incubation in the presence of unlabeled thymidine before extraction.

(d) *Fate of parental DNA after infection with strain* ts *12.1* c_1

Figure 2 shows that about one-fifth of the ^{32}P-labeled parental DNA is found in intermediate I. The parental DNA which has not entered intermediate I sediments at the same rate as the purified phage DNA marker. Some of the ^{32}P-radioactivity higher in the gradient than the marker position is degraded DNA already present in this phage preparation; it was observed in DNA extracted directly from phage particles. That there is no sign (Fig. 2(c)) of any ^{32}P-labeled material sedimenting slightly faster than the mature phage DNA even very late after infection indicates that strain *ts* 12·1 c_1 does not form intermediate II.

(e) *Fate of parental and pulse-labeled DNA after infection with strain* ts *5.1* c_1

(i) *Sedimentation through neutral sucrose gradients*

Neutral gradients (Fig. 3, frames 1, 2 and 3) show the accumulation of DNA in intermediate I by strain *ts* 5.1 c_1 as well as the failure to form intermediate II in considerable amounts. There may be a small amount of intermediate II (Fig. 3, frames 2 and 3), but the peaks are small compared to the same peaks in non-mutant strains at the same time in the latent period (Figs 3 and 5 of preceding paper). In no case is pulse-labeled DNA observed at the position of mature phage DNA. Control experiments showed that the ^{32}P-labeled DNA near the top of these gradients is indistinguishable in sedimentation rate from purified ^3H-labeled DNA extracted from phage particles. Thus, strain *ts* 5.1 c_1 fails to convert appreciable amounts of intermediate I DNA into intermediate II DNA.

(ii) *Sedimentation through alkaline sucrose gradients*

After infection with strain *ts* 5.1 c_1, intermediate I DNA can be followed during a chase without losing appreciable amounts of DNA through conversion to intermediate II. As with c_1 phage (preceding paper), much of the DNA does not sediment to the bottom in alkaline sucrose gradients (Fig. 3). Here, too, immediately after the pulse much of the labeled DNA appears to sediment in alkali like mature phage DNA. However, in the case of strain *ts* 5.1 c_1, the average sedimentation rate of the pulse-labeled DNA in alkali increases progressively during the chase, even though the neutral sucrose gradients show that this DNA is still in intermediate I (Fig. 3). The

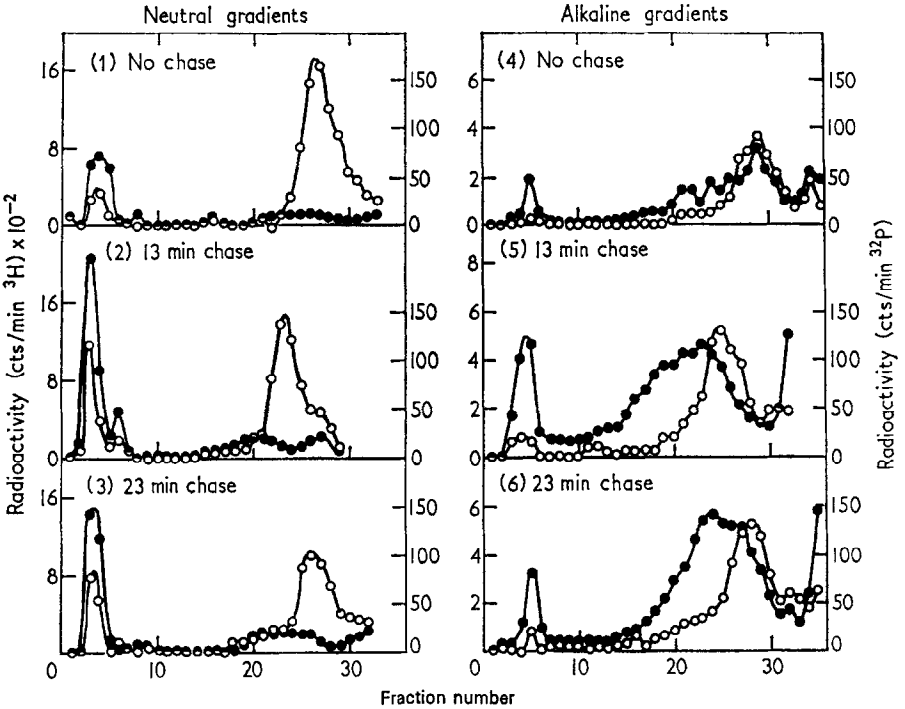

FIG. 3. Fate of parental and pulse-labeled phage DNA after infection with strain *ts* 5.1 c_1
The origin of the lysates is described in the legend to Fig. 1. Neutral gradients were centrifuged for 60 min at 35,000 rev./min at 23°C and alkaline gradients (preceding paper) were centrifuged for 90 min at 35,000 rev./min at 23°C. Recoveries were better than 75% in all cases.
(—○—○—) ^{32}P radioactivity; (—●—●—) ^{3}H radioactivity.

rate in alkali reaches almost twice the sedimentation rate of mature phage DNA by 33 minutes after infection (Fig. 3, frame 6).

(f) *Fate of parental and pulse-labeled phage DNA after infection with strain* ts 10.1 c_1

(i) *Sedimentation through neutral sucrose gradients*

Intermediate I is formed and disappears at similar rates after infection with strain *ts* 10.1 c_1 (Fig. 1(d)) and strain c_1 (Fig. 1(b)). In neutral sucrose, both parental [^{32}P]DNA and pulse-labeled [^{3}H]DNA from strain *ts* 10.1 c_1 (Fig. 4) behave in the same way as those from strain c_1 (preceding paper) at early times, but behave differently at late times. Almost all the pulse-label is first found in intermediate I, and then slowly moves into intermediate II during the chase. A corresponding amount of parental DNA appears in intermediate II, as indicated by the heavy shoulders on the ^{32}P peaks near the top of the gradients.

At late times with c_1 phage, more than 70% of the pulse-label is in mature phage DNA (preceding paper). In contrast, with strain *ts* 10.1 c_1 no more than 17% of the pulse-labeled DNA has been converted to the mature phage form (Fig. 4, frames 5 and 6).

With strain *ts* 10·1 c_1, there is some decrease in the average sedimentation rate of intermediate II during the latter part of the latent period. This apparent degradation could result either from some phage function acting on the structure of intracellular DNA or from asynchrony in the onset of lysis.

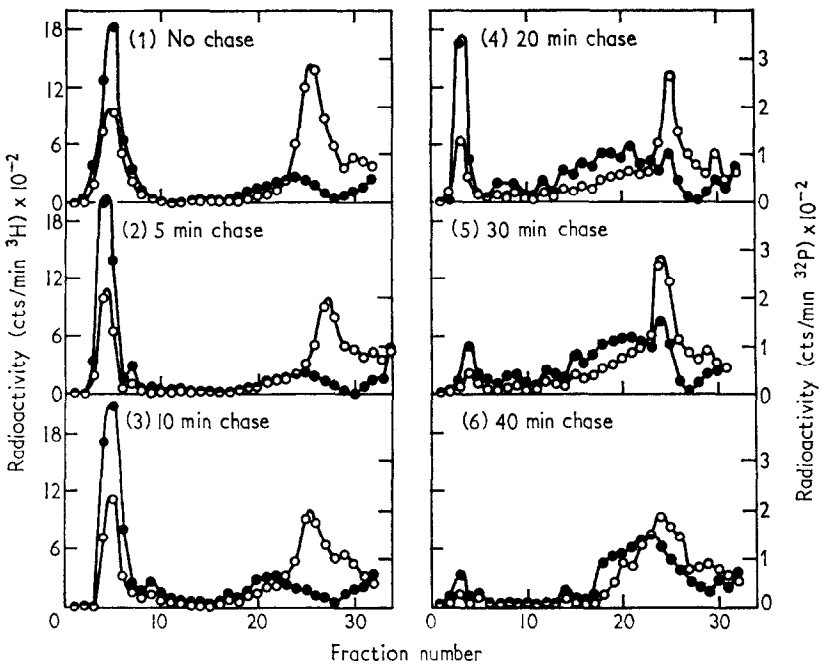

FIG. 4. Fate of parental and pulse-labeled phage DNA after infection with *ts* 10.1 c_1 phage: neutral sucrose gradients.

The origin of the lysates is described in the legend to Fig. 1. The gradients were centrifuged for 60 min at 35,000 rev./min at 23°C. Recoveries were better than 83% in all cases.

(—○—○—) ^{32}P radioactivity; (—●—●—) ^{3}H radioactivity.

(ii) *Sedimentation through alkaline sucrose gradients*

In neutral sucrose gradients, strain *ts* 10.1 c_1 does not appear to convert intermediate II DNA into mature phage DNA. In alkali, pulse-labeled DNA sediments 1·2 to 1·7 times more rapidly than the marker parental DNA in the middle of the latent period (Fig. 5), as is the case with c_1 phage (preceding paper). However, at the end of the latent period, a substantial amount of the pulse-labeled DNA sediments with the marker parental DNA (Fig. 5, frames 5 and 6), in contrast with the results obtained with neutral gradients. (Control experiments showed that the marker parental DNA sediments at the same rate as ^{3}H-labeled DNA extracted from phage particles.)

Even though at late times some of the pulse-labeled DNA from the *ts* 10.1 c_1 infection sediments with mature DNA in alkali, this mutant does not encapsulate any DNA. In a CsCl equilibrium density-gradient (Weigle, Meselson & Paigen, 1959) of the spontaneous lysate, there was no peak of radioactivity at or near the position marked by the viable phage (0·3 phage/cell). More than 90% of the radioactivity was recovered, spread throughout the gradient. However, if imperfect encapsulation of the DNA occurred, the DNA might remain sensitive to the nucleases liberated in the lysate. The activity of such nucleases was inferred from the fact that the radioactivity did not sediment to the bottom of the gradient, as anticipated if the radioactivity were still in polynucleotide chains of high molecular weight.

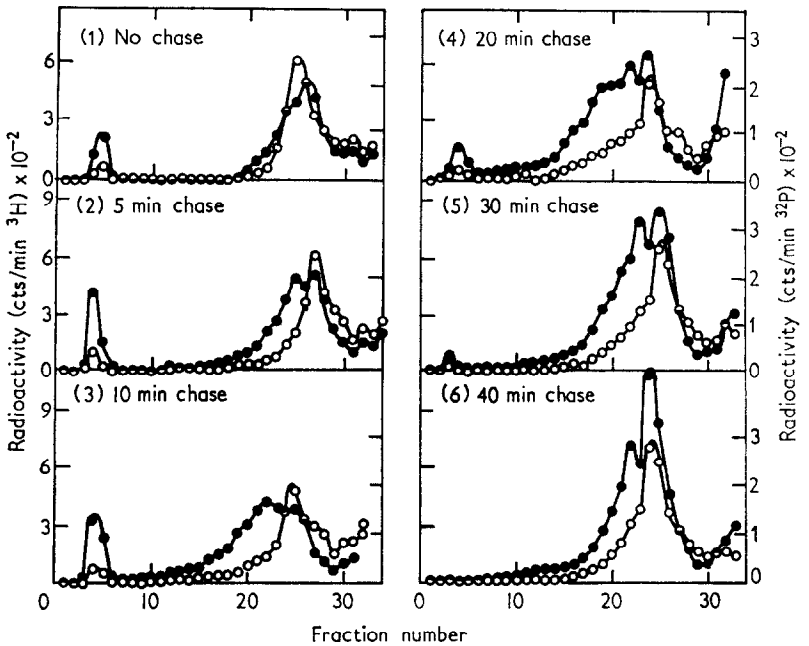

FIG. 5. Fate of parental and pulse-labeled phage DNA after infection with *ts* 10.1 c_1 phage: Alkaline sucrose gradients.

The origin of the lysates is described in the legend to Fig. 1. These lysates were applied to alkaline sucrose gradients as described in the preceding paper. The gradients were centrifuged at 35,000 rev./min for 90 min at 23°C. Recovery exceeded 77% in all cases.

(—○—○—) ^{32}P radioactivity; (—●—●—) ^{3}H radioactivity.

TABLE 3

Influence of the multiplicity of infection on the amount of parental DNA found in intermediate I

Multiplicity of infection (p.f.u.†/cell)	*Proportion of radioactivity at bottom* (%)		
	ts 5.1 c_1 phage (^{32}P-labeled)	*ts* 5.1 c_1 phage (^{3}H-labeled)	*ts* 12.1 c_1 phage (^{32}P-labeled)
0·5	23	—	17
1	18	29	16
2	21	12	18
5	24	19	16
10	17	32	18
20	22	25	17
40	—	30	—
50	20	—	16

Radioactive phage were used to infect strain 18 cells growing exponentially at 39°C in LCG20 medium. The multiplicities were calculated from previous determination (at 25°C) of the phage titer on strain 18 cells. The infected cells were incubated at 39°C for 30 min, after which they were lysed and the lysates centrifuged through neutral sucrose gradients with a cushion of high-density material at the bottom to ensure recovery (see preceding paper for experimental details). The gradients were centrifuged for 60 min at 35,000 rev./min at 23°C. The number of fractions/gradient varied from 27 to 33. Proportion of radioactivity in intermediate I was calculated by dividing the sum of the radioactivity recovered in the first 6 fractions by the total radioactivity recovered. The total recovery always exceeded 90%. The total radioactivity in any gradient was never less than 200 cts/min.

† Plaque-forming units on strain 18.

(g) *Influence of multiplicity of infection on the amount of parental DNA found in intermediate I after infection*

The multiplicity of infection has no effect on the proportion of parental DNA recovered in intermediate I with either of the strains that accumulate DNA in this form (Table 3). However, even at low multiplicities, most of the parental DNA (60 to 70%) does not enter intermediate I but remains at the marker position. This is due to the low efficiency of plating of P22 on this strain (strain 18) of *Salmonella* (Bezdek & Amati, 1967). On another strain (strain PV, from Dr B. Magasanik) where the efficiency of plating is three times higher, most of the parental DNA (63%) enters intermediate I, and the marker peak completely disappears.

4. Discussion

On the basis of the pulse-labeling experiments described in the previous paper, it was proposed that intermediate I DNA is a precursor of intermediate II DNA, which in turn is a precursor of the DNA in the progeny phage particles. The properties of the temperature-sensitive phage mutants described here support this scheme.

(1) Strain *ts* 12.1 c_1 fails to synthesize measurable amounts of phage DNA, but the parental DNA enters intermediate I. This means that intermediate I is not made up of DNA alone; even attached together, the unreplicated parental DNA molecules could not attain a sedimentation rate of 1000 S. It is likely that parental DNA combines specifically with other intracellular materials to form a complex which, upon extraction, is intermediate I. The binding of parental phage DNA into the complex would then seem not to be a consequence of DNA replication, but rather a prelude to DNA synthesis.

(2) Strain *ts* 5.1 c_1 accumulates intermediate I DNA. It is defective in the conversion of parental or newly-synthesized phage DNA into intermediate II or mature phage DNA. This indicates that intermediate I is a functional, as well as temporal, precursor of intermediate II DNA. Since *ts* 5.1 c_1 is a late mutant, the transfer of DNA from intermediate I to intermediate II would appear to be a late function, as was inferred earlier (preceding paper). The DNA in intermediate I sediments progressively more rapidly in alkali during the latent period. It is possible that whatever alteration in structure this represents precedes the formation of intermediate II in the normal case.

(3) After infection with strain *ts* 10.1 c_1, parental and newly synthesized phage DNA appear in intermediates I and II in the proper sequence. However, newly synthesized DNA does not appear as mature phage DNA, and both it and the parental DNA accumulate in a form closely resembling intermediate II in neutral sucrose gradients. This observation constitutes additional evidence that intermediate II is a precursor of the DNA in progeny phage particles. However, the sedimentation rate decreases near the end of the latent period. Even though this mutant fails to encapsulate any DNA, the intermediate II DNA may nevertheless be partially matured. This might explain why much of the pulse-labeled DNA eventually sediments at the same rate as mature phage DNA in alkaline gradients.

With strains *ts* 5.1 c_1 and *ts* 12.1 c_1, a constant proportion of parental DNA is found in intermediate I at multiplicities of infection from 0·5 to 50. There must be room for at least 50 phage genomes in the DNA-synthesizing complex, since comparison of strain 18 and strain PV with respect to efficiency of plating and proportion of parental

phage DNA found in intermediate I suggests that every phage DNA molecule which is competent to reproduce ultimately appears in intermediate I.

These results allow an elaboration of the scheme proposed in the preceding paper to describe the synthesis and maturation of phage P22 DNA.

(1) Parental phage DNA is injected into the host. All the competent DNA molecules become bound in a DNA-synthesizing complex which, upon extraction, is intermediate I. At least fifty phage DNA molecules can become bound. This process does not require the synthesis of measurable quantities of phage DNA.

(2) The phage DNA molecules are replicated while in the complex. Replication requires a normal gene 12 product. The structure of the replicated molecules is altered, and they sediment from 1·2 to 1·7 times as rapidly as mature phage DNA molecules in alkaline gradients. This alteration may well involve increasing the length of the polynucleotide chains. It is not clear whether the DNA molecules of altered structure replicate.

(3) The altered DNA molecules are released from the synthesizing complex and are now seen, after extraction, as intermediate II. The release of the DNA involves the product of gene 5.

(4) The intermediate II DNA molecules are again altered in structure to produce mature phage DNA. The gene 10 product is involved in this process. It is still not clear whether or not the production of mature phage DNA molecules from intermediate II is a consequence of the encapsulation process.

The synthesis and maturation of phage P22 DNA appears to involve an ordered sequence of changes in the structure of newly synthesized DNA. Although the exact structure of the intermediate forms and their physiological role in the biology of the phage is still uncertain, the outline of the process is clear.

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