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Genetic Expression in Heterozygous Replicative Form Molecules of ϕX174

V. MERRIAM,[1] L. B. DUMAS,[2] AND R. L. SINSHEIMER
Division of Biology, California Institute of Technology, Pasadena, California 91109

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Heterozygous replicative form molecules of bacteriophage ϕX174 deoxyribonucleic acid (DNA) have been constructed in vitro. These are composed of viral strands extracted from purified preparations of phage bearing *ts* mutations and complementary strands of either half length or full length synthesized with purified DNA polymerase, in vitro, on DNA from *am*3 phage. In infections with such heterozygous DNA, involving mutations in each of four different cistrons, phage with the genotype of the complementary strand comprised 1 to 20% of the total phage produced by a spheroplast population. From single-burst analysis of the progeny from DNA heterozygous in one cistron (B), it appears that those phage with the genotype of the complementary strand arise as major components in a small proportion of the infected cells rather than comprising a minor component in most cells. The implications of such a pattern of expression are discussed with respect to mechanisms of phage DNA synthesis.

The step-by-step description of nucleic acid synthesis during ϕX174 infection has formed a coherent picture (14). The infecting viral strand is converted to a double-stranded replicative form molecule (RF). This parental RF, i.e., that containing the infecting viral strand, is bound to a membrane site at which it is able to serve as template for ribonucleic acid (RNA) synthesis and to undergo semiconservative replication via the rolling circle mode. The complementary strand is elongated as synthesis of new complementary strands occurs on the double-stranded circle; a new viral strand is built on this elongated complementary strand (6, 12). The tail, now double-stranded and containing the original complementary strand from the parental RF and a newly synthesized viral strand, is released and closed into a circle as a daughter or progeny RF. If there are additional membrane sites available, this progeny RF may utilize them and become comparable to the true parental RF in function; if there are no additional sites available, this progeny RF remains in the cytoplasm, inert with respect to semiconservative replication (15) and perhaps to RNA synthesis (10; J. W. Sedat, Ph.D. Thesis, Calif. Institute of Technology, 1970). Later in infection, these progeny RF molecules participate in progeny viral single-strand synthesis.

[1] Present address: Department of Bacteriology, University of California, Los Angeles, Calif. 90024.

[2] Present address: Department of Biological Sciences, Northwestern University, Evanston, Ill. 60201.

The activities of the infecting viral strands have been extensively documented, whereas the activities of other components of the system have been inferred from population studies. In this study we have undertaken to study specifically the role of the parental complementary strand, i.e., the initial partner of the parental viral strand. For this purpose we have constructed heterozygous RF molecules containing a mutant viral strand and a wild-type complementary strand and used these to infect spheroplasts under both permissive and nonpermissive (with respect to the specific mutation) conditions.

The expected physical fate of the parental strands according to the model described above is shown diagrammatically in Fig. 1. The genetic fate is inferred from standard semiconservative replication. Thus, the initial replication of the RF leads to release of the parental complementary strand into an RF with genotype dictated by the complementary strand. In cells with a single replication site, this RF would remain inert in the cytoplasm until it entered into single-strand synthesis. All subsequent RF molecules produced would have the genotype of the parental viral strand, which remains at the replicating site. ϕX messenger RNA has the same sequence as the viral strand (9). Thus, phage-specific RNA synthesized prior to the first replication would be of the complementary genotype. If, as has been proposed, only the site-bound RF can be used as template for messenger RNA, then subsequent

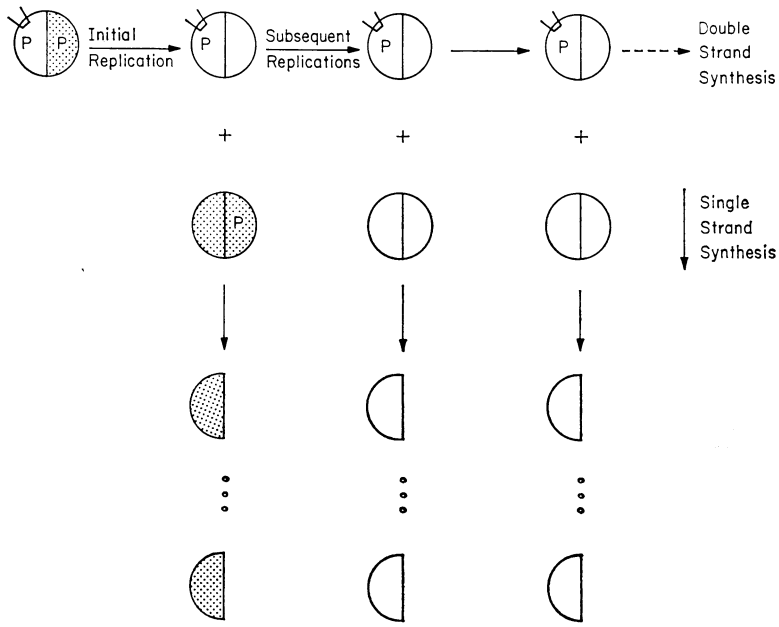

FIG. 1. *Diagrammatic representation of DNA replication in φX174. Circles represent RF molecules, the left hemisphere corresponding to the viral strand, the right to the complementary strand. The parental viral and complementary strands are those marked with P. The genotype of the complementary strand of the infecting parental RF is indicated by stippling.*

to the first RF replication the RNA synthesized would have the viral genotype.

One would predict then that most of the phage progeny of a heterozygous parental RF would be of the viral genotype, whereas a minor proportion of the phage would be of the complementary genotype. Under conditions nonpermissive for the viral strand, there may be significant rescue of the infection as a consequence of the presence of wild-type RNA formed very early in infection, but the distribution of the genotypes should not be radically different from the infection done under permissive conditions.

MATERIALS AND METHODS

Media. Cells for phage production or assay were grown in tryptone-KCl broth (2). Cells to be used for spheroplasts were grown in 3XD medium (8), and the infection was carried out in PA and PAM media (8). Deoxyribonucleic acid (DNA) and phage were stored in 0.05 M tris(hydroxymethyl)aminomethane (Tris), *p*H 8.0. SSC is 0.15 M NaCl and 0.015 M sodium citrate.

Phage and bacteria. φX174 mutants used were *am*3 (cistron E), *ts*4 (cistron H), *ts*79 (cistron G), *ts*9 (cistron B), and *ts*41D (cistron F). Grown under nonpermissive conditions, *am*3 phage provide increased burst sizes as a consequence of their failure to lyse the cells. Cistrons F, G, and H are responsible for structural components of the virus, whereas the function of the cistron B product is uncertain. There is evidence that cistrons F, G, and H may comprise an operon (R. M. Benbow et al., *in preparation*). Stocks were prepared in *Escherichia coli* C, a nonsuppressing host, at 30 C. Sucrose (10%) was added to the medium at the time of infection with the *ts* mutants to prevent lysis. After 2 hr, infected cells were concentrated by centrifugation and lysed on resuspension in 0.05 M borate buffer; the debris was removed by centrifugation. This procedure permitted increased titers and facilitated purification.

The phage were assayed on *E. coli* HF4714 (SuX), an *am* suppressor strain, at the permissive temperature of 30 C. All temperature-sensitive stocks were assayed for revertants by plating on SuX at 41 C. Stocks with revertant frequencies $\geqslant 10^{-5}$ were discarded. *E. coli* C, a nonsuppressor strain, was also used as indicator.

E. coli K12W6(*su*$^-$ *hcr*$^+$*rec*$^+$)was used for routine spheroplast assays. *E. coli* AB 2480(*su*$^-$*hcrA recA* 13) was used in spheroplast assays where indicated.

DNA preparation: viral strand. The temperature-sensitive phage were concentrated as described and the cell lysate was placed directly on a 5 to 20% sucrose gradient in 0.05 M borate. After centrifugation (SW25.1 rotor, 3 hr, 58,000 × *g*, 5 C), fractions were collected and assayed for phage. The peak fractions were pooled and dialyzed for 1 hr against 0.05 M Tris, *p*H 8.0. The phage suspension at 10^{12} to 10^{13} plaque-forming units per ml was then extracted once with water-saturated phenol and three times with ether; the ether was removed by bubbling with air at 37 C.

Complementary strand. The purified *am*3 phage suspension was extracted three times with phenol at 45 C and treated with ether and bubbling as above.

This material served as template in reactions with purified DNA polymerase; the complementary strand product was labeled with ^{32}P-labeled deoxyadenosine triphosphate (5, 7). Two classes of material were synthesized in separate experiments. (i) In the presence of excess initiator, the product at onefold synthesis consisted of segments of approximately one-half unit length as determined by sedimentation through alkaline sucrose. These are designated "random halves," as there is no reason to postulate specific initiation points. (ii) In the absence of added initiator, endogenous initiator activity permitted 0.33-fold synthesis. The product was primarily unit-length linear strands, designated here as "full-length linears" or simply "linears."

The viral strand template was removed by alkaline sedimentation (SW25.1 rotor, 0.1 M KOH, 24 hr, 58,000 $\times$ *g*, 5 C). One sedimentation of the random halves provided material containing only 10^{-5} viral circles (by weight) as measured by the infectivity of the preparation. With the linear strands, three successive sedimentations were required to reduce contamination to 10^{-3} (by weight). The final product was approximately homogenous but sedimented slightly more slowly than marker full-length linear strands. This is a consequence of repeated selection of the trailing edge of the linear strand region during purification.

The contamination by weight was ascertained by direct infectivity assay of the DNA preparations; it is therefore a measure of circular viral strands. Any linear viral strands purified along with the linear complementary strands would escape detection. On self-annealing, any circular or linear viral material could react with the linear complementary strands to form infectious RF. In all experiments, therefore, the background infectivity of the self-annealed complementary material was measured to determine the total contribution of contaminating "viral material." A correction for this contribution was subtracted from the results attained with the heterozygous RF molecules before calculation of the expression of the complementary strand.

Annealing. DNA samples in 0.05 M Tris, *p*H 8.0, were mixed at concentrations between 10^{11} and 10^{12} strands per milliliter (0.3 to 3 μg/ml), and 24X SSC was added to give a final concentration of 2.4X SSC. The solution was incubated at 67 C for 1 hr; longer incubation (up to 15 hr) gave no change in the amount of complementary strand expression. Under these incubation conditions, single-stranded DNA circles sustain 0.1 hit/hr, as determined from infectivity loss.

Biological assays. Phage were assayed in the standard manner. The infection of spheroplasts was carried out according to Guthrie and Sinsheimer (8). Two dilutions of each sample were always included to insure that the phage yield was linear (on a log-log plot) for the DNA concentrations used. Progeny phage were assayed at 30 and at 41 C. The ratio of the number of phage plating at 41 C (corrected for the yield from infections initiated by the self-annealed complementary strand material) to the number of phage plating at 30 C is defined as the expression of the complementary strand. Correction of the 30 C plating is appropriate, but insignificant.

When yields were to be examined under nonpermissive conditions, the DNA and spheroplasts were mixed and incubated for the initial 15 min at 41 C. Prewarmed (41 C) PAM was added; half of the mixture was incubated at 41 C, and half was shifted down to 30 C. This pretreatment did not reduce the yield of either wild-type or temperature-sensitive phage formed after shift-down. After 2 to 3 hr, the spheroplasts were lysed by 10- to 100-fold dilution into 0.05 M borate buffer.

For single-burst analysis, the DNA was diluted in 0.05 M Tris, *p*H 8.0, and mixed with an equal volume of spheroplasts. An appropriate amount of PAM was added, and two drops of the solution were added from a 5-ml pipette (0.1-ml portions) to each of 100 to 200 tubes. All unnecessary shear was avoided to maintain the integrity of the spheroplasts. After 2 hr of incubation at the desired temperature, the spheroplasts were lysed by the addition to each tube of 0.4 ml of 0.05 M Tris, *p*H 8.0. The whole sample was plated on *E. coli* SuX at 30 C. All progeny will plate under these conditions. After 6 hr all plaques were picked with sterile toothpicks and streaked on SuX at 41 C (plates prewarmed to 37 C) to determine temperature sensitivity and on *E. coli* C at 30 C to determine whether they were amber. For such analysis, it is critical to use young plaques, that is before diffusion can lead to cross-contamination of the plaques, and to have warm plates for the temperature-sensitivity analysis.

In the single-burst experiments, a Poisson distribution of infected centers in the tubes is assumed. The fraction of plates without any plaques is therefore used to calculate the average number of infected centers per sample. Only series with initial concentrations of <0.2 infected centers per portion were analyzed. The ratio of DNA strands to spheroplasts to give appropriate concentrations of infective centers was 1/200 at 41 C and 1/6,000 at 30 C. The actual concentrations of DNA are on the order of 10^6 strands/ml at 41 C and 10^5 strands/ml at 30 C, with the actual concentration varying somewhat for different spheroplast preparations.

RESULTS

Annealing of viral and complementary strands. The small complementary strands of ϕX174 have a C_0t number of 5×10^{-3} moles per liter per second (1) and therefore anneal rapidly even at low concentrations. At equal input of the complementary and viral strands, there is a rapid initial coalescence to yield a double-stranded molecule with no physically detectable, free, single-stranded material remaining. A slower registering within this double-stranded molecule, which has some single-stranded properties, leads to a product indistinguishable by sedimentation velocity or neutral buoyant density from native RF II, the circular double-stranded molecule containing single-strand breaks in one or both strands. The

infectivity of the single-strand mixture drops by 10- to 40-fold during annealing; the RF II isolated from infected cells is typically 1/20 as infectious as the same number of single-stranded circles.

Concentration dependence of expression. Activity, or expression, of the complementary strand will be defined in terms of its ability to cause the appearance of phage of the complementary strand genotype in an infection initiated by a heterozygous RF. Numerically it is the proportion of the phage in the burst which plate at 41 C (*see below*). With no guidelines for prediction, one might expect such expression to be relatively independent of the cistron examined and to be maximal at the equivalence point of one gene added in the complementary material for each gene present in the viral material.

Viral DNA was mixed with various amounts of complementary strand in 2.4X SSC and annealed at 67 C for 1 hr, a time sufficient to yield RF identical to native RF by the criteria listed above. This DNA was then used, without further purification, to infect spheroplasts at 30 C for 2 hr. At the end of this time, the spheroplasts were lysed by dilution and the progeny were assayed at 30 and 41 C. The heterozygous RF molecules have the genotype represented as v(+ *ts*x)/c(*am*E +), i.e., the viral strand is temperature-sensitive in cistron *x*, wild-type in all other cistrons; the complementary strand is amber in cistron E, wild-type in the remainder of the cistrons, in particular in *x*. Thus, those phage plating at 41 C either represent revertants in the viral strand or have arise in some way from the information of the complementary strand. In all cases, the revertant frequency for the viral strand is $< 10^{-5}$. The contribution from the self-annealed complementary strand material is more significant, amounting to 1 to 10% the values from infections initiated by the heterozygous RF. All values are therefore corrected for the number of phage produced by the self-annealed complementary material. The fraction of phage plating at 41 C, after this correction, compared to the total phage plating at 30 C, is defined as the expression of the complementary strand. The activity of the complementary strand is therefore defined here only in terms of progeny generation; no implication regarding RNA formed during infection is made.

Figure 2 shows the dependence of expression for cistrons G and B on the relative number of the complementary strand equivalents. Maximum expression does not occur at a 1:1 input; expression actually increases with increasing complementary strand input in both cistrons examined to 10-fold that observed at the equivalence point, whether the complementary random halves or linears are used. A 10-fold difference in expression in these two mutant cistrons is apparent at all levels. To extend this study, expression was examined for four cistrons at a 1:1 input by

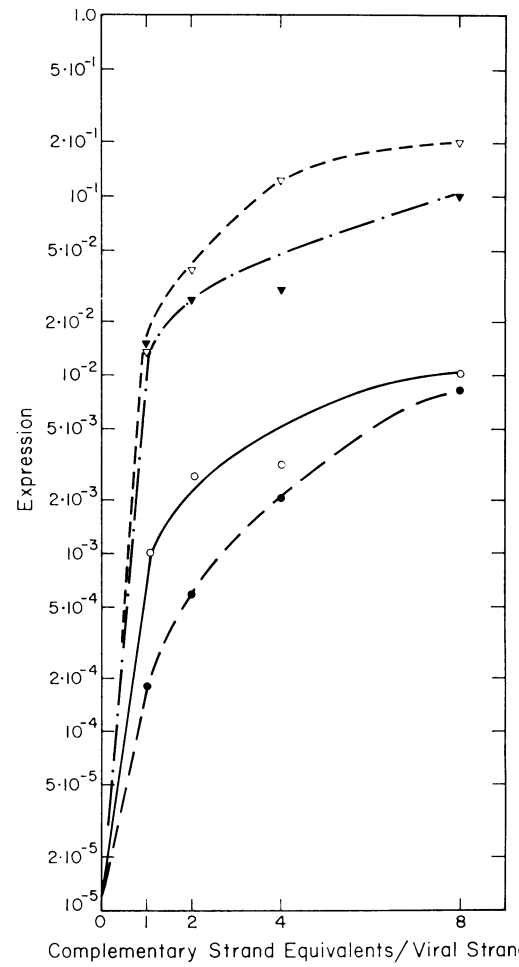

FIG. 2. *Expression in heterozygous RF molecules as a function of the relative number of viral and complementary strands in the annealing mixture. Viral strands extracted from purified temperature-sensitive phage (cistrons G and B) were mixed at ~10^{11} strands/ml in 2.4X SSC with amounts of complementary strand material equivalent to 1, 2, 4, and 8 times the viral strand present. Both random half and linear complementary strands were used. Each mixture was incubated at 67 C for 1 hr, diluted with 0.05 M Tris (pH 8.0), and used to infect spheroplasts. Expression of the complementary strand is defined by the ratio of the number of phage plating at 41 C (after correction; see Materials and Methods) to the total phage plating at 30 C. Symbols:* ○, *cistron G, complementary strand linear;* ●, *cistron G, complementary strand halves;* ▽, *cistron B, complementary strand linear;* ▼, *cistron B, complementary strand halves.*

weight (Fig. 3a and b). For the random halves (Fig. 3a), expression is significantly lower in cistrons F, G, and H than in cistron B; with the full-length linears (Fig. 3b), the average values show less variation although the range of values in different assays is greater. The source of this variation, which is greater between samples than within samples, is unknown.

Thus, neither expectation (the maximum expression at equivalence and equivalent expression of all cistrons) was realized. The comparison of expression for random halves and linears in the four cistrons suggests that all portions of the genome are not completely comparable under these conditions.

Activity of the complementary strand under nonpermissive conditions. If the infected spheroplasts are incubated at 41 C, cells in which only temperature-sensitive protein appears do not yield phage. Thus, the phage yield for single-stranded DNA from phage bearing a *ts* mutation drops at 41 C by a factor of 10^3. In infections by v(+ *ts*B)/c(*am*E +) where the expression of cistron B under nonpermissive conditions is sufficient to give yields significantly greater than the background from temperature-sensitive DNA alone, the plaques may be analyzed individually. The absolute number of *ts*-mutant phage in a 41 C lysate from spheroplasts infected by a v(+ *ts*B)/c(*am*E +) exceeds that expected from the *ts*-mutant DNA alone by 10- to 100-fold and comprises 13% of the plaques analyzed. It appears that the wild-type complementary strand in the parental RF may then rescue the infection, probably through the formation of wild-type messenger RNA prior to the first replication of the parental RF.

Importance of repair to expression. If all expression in heterozygotes arises from random repair of one strand to yield orthoduplexes of the two possible types (3), expression should be greatly suppressed in hcr⁻rec⁻ spheroplasts. Heterozygotes of the four cistrons were used to infect AB 2480 (rec 13 hcrA) and K12W6 (rec⁺-hcr⁺). As shown in Table 1, expression in all cases is comparable in the two hosts. Repair can then play only a minor role in expression.

Single-burst analysis. The fact that expression is essentially the same in hcr⁻rec⁻ cells as in hcr⁺rec⁺ cells indicates that the entity responsible for expression of the complementary genotype is a heterozygous RF molecule. As outlined in the introduction, one may make predictions as to the composition of a burst from a single cell infected with such a molecule. The data thus far presented have concerned only total populations of phage resulting from the bursts of many cells. Repair occurring in some cells could distort the apparent pattern of expression, notably for the RF molecules containing complementary material of less than full length, that is the random halves. Some cellular conversion of one or two half-length pieces to a full-length linear complementary strand is obviously necessary. If only one

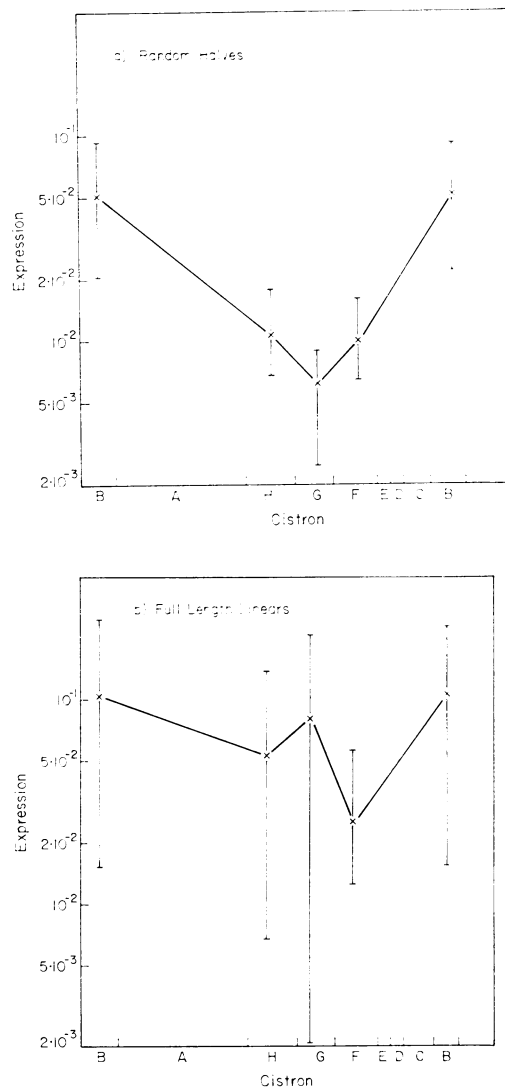

FIG. 3. *Expression in heterozygous RF molecules in cistrons B, F, G, and H. Viral DNA from purified temperature-sensitive phage (cistron B = ts9; F = ts4; G = ts79; H = ts41D) was mixed at 10^{11} strands/ml with 10^{11} strand equivalents of wild-type complementary strand of (a) half length or (b) full length. Expression is defined as in Fig. 2. Lines indicate the range of values found in a number of assays; the average values are indicated and joined. Abscissa distances represent the estimated relative sizes of the cistrons (Benbow et al., in preparation).*

TABLE 1. *Effect of repair on expression in heterozygous RF molecules*[a]

Cistron	Expression in	
	hcr⁺rec⁺	hcr⁻rec⁻
F	0.061	0.019
G	0.066	0.100
H	0.026	0.014
B	0.082	0.140

[a] Heterozygous RF molecules, prepared at a 10:1 ratio of complementary linear strand to viral strand, were assayed in K12W6 spheroplasts (hcr⁺rec⁺) and in AB2480 spheroplasts (hcr⁻rec⁻). Values in the rec⁻hcr⁻ host are corrected for the relative efficiency of control single-stranded DNA in the two hosts.

piece were retained, RF molecules of genotype v(+ *ts*x)/c(*am*E tsx) and v(+ *ts*x)/c(+ +) may result in addition to the expected v(+ *ts*x)/c(*am*E +). Site-specific changes may also occur for less obvious reasons with the full-length linear strands. It is clear that a more profound analysis would result from examination of bursts from single infected spheroplasts. In addition, the cistron E marker in the complementary strand material, which has been mentioned but not utilized to this point, will provide additional insight into intracellular events. The general prediction is that in bursts of single spheroplasts the progeny corresponding to the viral genotype should be in excess and that most bursts will include a small number of progeny with the complementary genotype.

Single bursts of infections initiated with heterozygous RF molecules of genotype c(+ *ts*B)/c(*am*E +) containing random halves or linear complementary strands were examined under both permissive and nonpermissive conditions as described above. The burst sizes of these spheroplasts were extremely variable, both from control single-stranded DNA infections and from heterozygous RF infections. The average burst in both cases was approximately 40 phage per infected spheroplast; the range was from 1 to 200 phage per burst.

The genetic analyses of the single bursts appear in Table 2. Though mixed bursts are observed with both types of RF, under both permissive and nonpermissive conditions, in all cases the single genotype bursts are in excess. Under permissive conditions, the majority of cells yield phage only of the viral genotype, + tsB. Such bursts would arise if all of the complementary strand material were eliminated by the cell prior to RF replication. Under nonpermissive conditions, none of these bursts should be observed; however, there is rescue of such bursts in the case of heterozygous RF molecules containing linear complementary strands. The presence of the complementary strand may therefore be inferred in such cells (although it does not appear in the progeny) and thus, by extrapolation, in some of those cells producing only viral genotype bursts under permissive conditions.

As the burst-size distribution is comparable for spheroplasts yielding single or mixed genotype bursts, it may be concluded that the 10 to 20% expression of the complementary strand character in the cistron B region under permissive conditions arises from 10 to 20% of the cells yielding all or mostly complementary type phage, rather than most of the cells yielding 10 to 20% complementary type phage and 80 to 90% viral type phage.

Most bursts under nonpermissive conditions contain the selected (+) complementary strand marker in the cistron B region. In the unselected cistron region, the marker from the complementary material, *am*E, is present in combination with the complementary strand cistron B region (+) in 9 of 29 cases in the progeny of RF containing random halves and in 14 of 25 cases in the progeny of RF containing linears. There is thus greater linkage between the cistron E and cistron B region when the complementary strand is of greater length. This observation is in accord with the proposal that some DNA elimination occurs in the RF molecules containing several random halves per complementary strand.

A significant portion of the bursts under all conditions is mixed. Those appearing under permissive conditions might be attributed to tubes containing more than one infected center; the number appearing under nonpermissive conditions is much too great to be so explained and may be assumed to arise from a single spheroplast. In most of these bursts the cistron marker from the complementary strand is in excess. In total, these data indicate that in those bursts where phage with the genotype of the complementary strand occur at all, they comprise all or most of the population of the burst.

DISCUSSION

The current model for DNA synthesis in φX174-infected cells is outlined in the introduction. Such a description is consistent with the apparent failure of direct parental-to-progeny transfer and the requirement for the integrity of the parental viral strand late in wild-type infection. The role of the parental complementary strand in this model is quite subsidiary. If the parental RF is heterozygous, as described here,

TABLE 2. *Single-burst analysis of spheroplasts infected with heterozygous RF molecules of type v(+ tsB)/c(amE +)*[a]

Burst composition	No. of bursts			
	Spheroplast incubation (30 C)		Spheroplast incubation (41 C)	
	Complementary random halves	Complementary linears	Complementary random halves	Complementary linears
Single genotype				
+ *ts*B	42	44	1	13
*am*E +	8	2	5	7
+ +	1	5	16	8
Two genotypes			(9, 3)	
	(1, 47)		(6, 1)	(38, 11)
(*am*E +) (+ *ts*B)	3 (7, 43)		4 (2, 1)	3 (65, 4)
	(45, 5)		(5, 3)	(5, 1)
			(13, 2)	(93, 22)
(+ +) (+ *ts*B)	1 (38, 12)	2 (56, 8)	4 (18, 5)	3 (117, 3)
		(3, 2)	(3, 1)	(1, 1)
			(30, 6)	
		(74, 20)		(152, 8)
(*am*E +) (+ +)		3 (21, 11)		4 (31, 131)
		(1, 1)		(50, 4)
				(34, 18)
Total bursts analyzed	55	56	30	38

[a] An RF sample containing 1:1 viral and complementary strand material was diluted such that standard treatment during the spheroplast assay yielded a solution containing < 2 infected spheroplasts/ml. An equal volume of spheroplasts was added to a portion of the diluted RF solution and the mixture was incubated at 41 C for 15 min. Eight volumes of PAM were added, and the sample was quickly distributed in 0.1-ml samples (< 0.2 infected centers/tube) prior to 2 hr of incubation of the spheroplasts at 30 C or at 41 C. The infected cells were then lysed osmotically by addition to each tube of 0.4 ml of Tris, *p*H 8.0. The entire tube was plated on *Escherichia coli* SuX at 30 C to permit all progeny to plate. After 6 hr of incubation, all plaques were picked and tested on SuX at 41 C and on *E. coli* C at 30 C. Wild-type phage in the cistron B region will plate in the former case; wild-type phage in the cistron E region will plate in the latter. By direct examination one may therefore describe the entire genome of each phage in the burst. Bursts were identified as containing a single genotype or more than one genotype and so recorded. Figures in parentheses for mixed bursts give the actual numbers of the two phage types observed in the mixed bursts in the order indicated. The totals represent pooled data from a number of similar experiments done at different times. In any given experiment, the $P(O)$ was >0.8, for an average of <0.2 infected centers/tube.

the wild-type complementary strand would be released in the first daughter RF molecule. It could then express itself genetically only in single strands made from that RF. It could be expressed phenotypically through RNA synthesis prior to RF replication. Under conditions nonpermissive to the viral strand, this RNA might be adequate to effect some rescue of the infection.

It is thought that most, if not all, spheroplasts have only one site for DNA replication. Genetic recombination is observed but not complementation (11; C. A. Hutchison III, Ph.D. Thesis, California Institute of Technology, 1969). The actual degree of RF replication within a spheroplast has not been measured. If only a single replication of parental RF occurs, the viral type RF would remain at the membrane where it might not participate in progeny synthesis. The only daughter RF would then be of complementary genotype and only complementary type phage would appear in the burst. Additional replication of the parental RF would produce additional viral-type RF molecules and would be expected to reduce the relative contribution of the complementary genotype RF to the pool. The proportion of complementary genotype RF molecules would drop as a function of the replication of the parental RF and presumably so would the proportion of progeny of complementary strand genotype.

The essential result obtained in these experiments is the expression of the genotype (at the *ts* locus) of the complementary strands in infections with heterozygous RF. This expression is at least one order of magnitude greater than can be explained away as a consequence of gene reversion or homozygous RF contamination. It does not

seem to be at all dependent upon the presence or absence of a host cell DNA repair system (3, 13).

The expression of complementary strand genotype is quantitatively significant. In all cases the progeny from a population of spheroplasts infected with heterozygous RF molecules contained between 1% and 10% phage with the genotype of the complementary strand. It has been observed with mutants in each of four different cistrons. It is observed equally well whether the complementary strands are present as approximately viral DNA length pieces or half-viral DNA length pieces.

That the genotype of the complementary strand is present in only a minority of the progeny would be expected from the model. However, examination of single bursts of infected cells disclosed a pattern far from expected. Rather than most cells yielding mixed bursts with a few complementary type phage, the majority of infected cells yield progeny exclusively of the viral genotype (i.e., *ts*). Only a small class of cells yielded bursts with phage of complementary genotype; these contained in many cases progeny of exclusively complementary genotype.

In some of those cells which yielded only viral type phage, the presence at some stage of the complementary strand may be inferred from the rescue of these bursts under conditions nonpermissive for the viral strand phenotype. In the majority of such cells, however, it is at least possible that the complementary strand may have been eliminated.

Various hypotheses may be advanced to account for these observations. As recombination is observed in spheroplasts, it is evident that more than one RF can penetrate these bodies. The failure to observe complementation suggests a limitation upon templates for transcription, but this is not necessarily true for replication. The structures of the products of viral and complementary strand annealing used in these experiments are not known. Thus, in some instances a viral strand may be annealed to different portions of two complementary strands (or vice versa). These possibilities, combined with severely limited RF replication and grossly unequal use of one or two out of a small number of RF molecules as templates for progeny DNA synthesis, could give rise to the observed results.

Another explanation would involve the complete failure of RF replication in a fraction of cells. In this case all progeny virus but one (which could occasionally be missed) would be of complementary strand genotype.

A more interesting explanation would allot a more significant role to the complementary strand in the determination of progeny virus genotype than is expected from the model previously developed. Such experiments as those recently performed (V. Merriam et al., *in press*), in which the decay of a tritium atom attached to the 5-carbon of cytosine in the viral strand of a parental RF was unable to induce mutation, whereas the decay of a similar tritium atom in the complementary strand of a parental RF was markedly effective, are also suggestive of an assignment of much greater significance for the complementary strand of the parental RF in the determination of progeny genotype.

The results recently described by Dressler and Wolfson (4) led them to propose a model of ϕX RF replication via elongation of the viral strand. Such a model would give the complementary strand the master role in the determination of progeny genotype. This model is in direct contradiction to one proposed earlier (13) derived from a different line of evidence. Unfortunately, the experimental data presented by Dressler and Wolfson do not unambiguously resolve the issue; there are two molecular products from each DNA replication and the interpretation of their data depends upon an assumption as to which product is observed in their studies.

Adoption of such an alternative model would then require a special explanation, such as elimination of the complementary strand, for the majority of spheroplasts in which the viral strand genotype is dominant among the progeny.

It is conceivable that the first daughter RF is distinct from subsequent daughter RF and shares with the parental RF the responsibility for RF replication. The relative involvement of the two might vary, dependent upon external factors.

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