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Bacteriophage T7 morphogenesis and gene *10* frameshifting in *Escherichia coli* showing different degrees of ribosomal fidelity

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Summary. Bacteriophage T7 infection has been studied in *Escherichia coli* strains showing both increased and decreased ribosome fidelity and in the presence of streptomycin, which stimulates translational misreading, in an effort to determine effects on the apparent programmed translational frameshift that occurs during synthesis of the gene *10* capsid protein. Quantitation of the protein bands from SDS-PAGE failed to detect any significant effects on the amounts of the shifted 10B protein relative to the in-frame 10A protein under all fidelity conditions tested. However, any changes in fidelity conditions led to inhibition of phage morphogenesis in single-step growth experiments, which could not be accounted for by reduced amounts of phage protein synthesis, nor, at least in the case of decreased accuracy, by reduced amounts of phage DNA synthesis. Reduction in phage DNA synthesis did appear to account for a substantial proportion of the reduction in phage yield seen under conditions of increased accuracy. Similar effects of varying ribosomal fidelity on growth were also seen with phage T3, and to a lesser extent with phage T4. The absence of change in the high-frequency T7 gene *10* frameshift differs from earlier reports that ribosomal fidelity affects low-frequency frameshift errors.

Key words: Frameshifting – Phages T7, T3, and T4 – Ribosomal fidelity – Translational accuracy – Streptomycin

Introduction

Bacteriophage T7 gene *10A* encodes a 344 amino acid protein (36414 daltons; excluding the processed N-terminal methionine), which is the major capsid protein of the phage head. Another capsid protein, *10B* (~ 41800 daltons), is made in lesser amounts (~10% of 10A). Various lines of evidence have suggested that 10B arises from a −1 translational frameshift occurring at a run of 4 U residues spanning codons 340–341 (excluding the AUG initiation codon) of *10A* mRNA, thus adding an additional 53 amino acids at the carboxyl-end of the protein (Dunn and Studier 1983). Direct analysis of the 10A and 10B proteins by peptide mapping and partial sequencing of a peptide spanning the junction of the two proteins have now confirmed the frameshift (Sipley et al. 1991); furthermore, the complete sequence of this "junction" peptide has, in fact, pinpointed the frameshift site to codons 340–341 of *10A* (Condron et al. 1991).

Previous studies involving instances of low-level translational frameshift errors indicated that *rpsL* mutations (in ribosomal protein S12 of the 30S subunit), which confer resistance to the antibiotic streptomycin (Sm^r), decrease frameshifting in the absence of streptomycin (Atkins et al. 1972; Weiss and Gallant 1983). The addition of streptomycin, which stimulates translational misreading, increased frameshifting in Sm^r strains, as did the introduction of a ram (*r*ibosomal *am*biguity) mutation (*rpsD*, ribosomal protein S4 of the 30S subunit) into a host containing a highly restrictive *rpsL* allele (Atkins et al. 1972). Additional studies with *rpsL* mutants have shown a reduction in the variety of translation-specific errors in vivo and in vitro (as reviewed in Gorini 1974; and more recently Parker 1989). The increased accuracy phenotype of *rpsL* mutants has been attributed to an increased efficiency of translational-proofreading, compared to wild-type (wt) ribosomes (Ruusala et al. 1984); *rpsD* mutants with the ram phenotype exhibit decreased translational accuracy or increased misreading, similar to the effect of addition of streptomycin to wt ribosomes. In vitro experiments suggest that *rpsD* ribosomes are inefficient in translational proofreading (Andersson and Kurland 1983) and it has been suggested that streptomycin similarly causes misreading with wt ribosomes by reducing the efficiency of translational proofreading (Ruusala and Kurland 1984).

Some level of frameshifting must continue to occur

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even in highly restrictive *rpsL* mutants, since at least two essential genes of *Escherichia coli* (*dnaX* and *prfB*) generate their products by programmed translational frameshifts (as pointed out by Parker 1989, for *prfB*). Nevertheless, it seemed possible that conditions of altered fidelity might significantly change the frequency of frameshifting, particularly in the light of the earlier studies showing effects of fidelity manipulations on low-level frameshift errors. In this study, we examine the effects of different host translational fidelity mutations on the high-frequency bacteriophage T7 gene *10* − 1 frameshift, using a set of well-characterized ribosomal fidelity mutants in an F^- isogenic background. We find the frameshift to be unaffected by a hyperaccurate *rpsL* allele, a decreased accuracy *rpsD* ram mutation, or the presence of the translational misreading drug streptomycin. Unexpectedly, however, we find significant inhibitory effects on T7 morphogenesis under these conditions of altered translational fidelity.

Materials and methods

Strains, plasmids, and bacteriophages. Table 1 summarizes the relevant characteristics of the strains used in this study. *E. coli* K12 derivative XAc (F^-, *rpsD*$^+$, *L*$^+$) is the host strain for the ribosomal fidelity alleles *rpsD14* (strain UL478) and *rpsL1204* (strain UL435), obtained from Dr. Leif Isaksson, Stockholm, Sweden. These alleles have been extensively tested with respect to their fidelity characteristics, and shown, respectively, to decrease or increase translational accuracy (Olsson and Isaksson 1979; Andersson and Kurland 1983; Ruusala and Kurland 1984; Kirsebom and Isaksson 1986; Faxén et al. 1988). Furthermore, in our hands, the *arg* auxotrophy in strain XAc could be suppressed by the *rpsD14* allele; this auxotrophy could also be phenotypically suppressed by addition of streptomycin, but not in the presence of the *rpsL1204* allele. Strain 011′ was used as a standard host for growth of T7 wt phage and T7 gene *9am17* (amber 17 in gene *9*) phage stocks. Strain B401 (amber Su^+) was the host for growth of T7 gene *10am*13 (amber 13 in gene *10*) phage stocks. Strain XAc was used as the standard host for growing phage stocks of wt T3 and T4. Preparation of phage stocks was similar to that described for other phage (Goldman and Lodish 1973). T-broth (Studier 1969; 1% tryptone, 0.5% NaCl) plus M9 supplements (Miller 1972) was the standard growth medium for preparation of phage stocks.

The XAc strains bearing the ribosomal fidelity alleles were streaked to obtain single colonies and grown at 37° C on M9 plates supplemented with 0.2% glucose, 5 μg/ml thiamine, 0.6 mM arginine, and 2 mM proline. Parallel cultures on M9 medium were grown from single colonies. Colonies of the streptomycin-pseudodependent strain, Sm^p, were grown as above on M9 plates supplemented with 500 μg/ml dihydrostreptomycin (Sigma). Cultures of the Sm^p strain when grown without streptomycin (Sm^p without Str), were started from the streptomycin-containing plates; however no streptomycin was added to the broth culture. Under these conditions the Sm^p strain usually required at least 12 h to reach 2×10^8 cells/ml.

Table 1. Strains used

Strain, plasmid, or phage	Relevant characteristics	Source and/or reference
Escherichia coli strains:		
XAc	F^- *ara argE Δ(lac-proB) nalA rif thi*	Coulondre and Miller 1977
UL478	As XAc but *rpsD14*	Olsson and Isaksson 1979
UL435	As XAc but *rpsL1204*	Faxén et al. 1988
011′	Su_1 *thi* Sm^r *gal*	Studier 1969
B401	Su^+ (UAG)	F.W. Studier
BL21	(DE3)λ lysogen (T7 RNA polymerase)	Studier et al. 1990
Plasmids:		
pAR3625	T7 gene *10* clone, amp^r	Studier et al. 1990
pLysS	T7 lysozyme clone, cm^r	Studier et al. 1990
Phage:		
T7	wild type	J. Dunn
T7 *9am17*	amber 17 in gene *9*	J. Dunn
T7 *10am13*	amber 13 in gene *10*	F.W. Studier
T3	wild type	W. McAllister
T4	wild type	L.S. Ripley

Analysis of T7 DNA packaging signal. Parallel cultures of wt, ram, and streptomycin-pseudodependent strains were grown and infected with T7 wt as described, except that the growth medium was B2 plus 5XP (Studier 1975). Upon reaching 2×10^8 cells/ml, 2 ml of each culture were removed prior to infection and mixed with 1 ml 3 × lysis buffer comprising 30 mM NaCl, 30 mM EDTA, 30 mM TRIS-HCl, pH 8.1, 1.5% SDS. The lysate was then frozen in a dry ice-acetone bath. Immediately following this zero timepoint (uninfected sample), T7 phage (5 pfu/cell) were added to the remainder of the culture and duplicate 2 ml samples were removed at the indicated times, mixed with 3 × lysis buffer, and frozen as described above for the uninfected cells. Samples were thawed at 37° C, and 15 μl proteinase K (10 mg/ml) was added to give a final concentration of 50 μg/ml. After incubation at 37° C for 90 min, 0.45 ml aliquots were transferred to 1.5 ml microfuge tubes, mixed with 50 μl 3 M sodium-acetate, pH 6.0, and 0.5 ml isopropanol. The samples were incubated at −20° C for 1 h and then centrifuged for 5 min. The resulting precipitates were washed with 1 ml 70% ethanol, dried under vacuum, and then resuspended in 200 μl 10 mM TRIS-HCl (pH 8.0), 1 mM EDTA (TE buffer); 100 μl of 7.5 M ammonium acetate was added followed by addition of 0.6 ml ethanol. Samples were placed at −20° C for 1 h, centrifuged, and the pellets washed with 70% ethanol. After drying in vacuo, each sample was dissolved in 20 μl TE buffer and mixed with an equal volume of 2 × restriction buffer (1 × is 10 mM TRIS-HCl, pH 7.9, 8 mM

$MgCl_2$, 100 mM NaCl) containing 0.2 units each of *Nru*I, *Bgl*I, *Nco*I, and *Sal*I. This mixture of enzymes yields a characteristic pattern of seven bands from mature phage T7 DNA and two additional bands from replicating T7 DNA: one representing the concatemer junction and the other a covalently closed hairpin that originates from initiation of asymmetric DNA replication near the right end of the T7 DNA molecule (Chung et al. 1990; J. Dunn, unpublished results). Samples were digested overnight at 37° C and then treated with pancreatic RNase and RNase T1 (10 μg/ml each for 15 min) prior to electrophoresis in a 1% agarose gel. Gels were stained with 0.5 μg/ml ethidium bromide and photographed with ultraviolet illumination.

Protein synthesis during T7 phage infection of cells under conditions of altered ribosomal fidelity. Cultures at 2×10^8 cells/ml were infected with T7 phage at a multiplicity of 5 pfu/cell. At the indicated times, the cultures were pulse-labeled with [^{35}S]methionine (18 Ci/mmol, 12 μCi/ml) for 2 or 3-min intervals. Aliquots (10 μl) were removed to determine label incorporation by base treatment and trichloroacetic acid (TCA) precipitation with bovine serum albumin (BSA) as a carrier. Labeling was terminated by the addition of NaOH (0.2 N) to the sample followed by incubation for 10 min at 37° C. The proteins were precipitated on ice with 10% TCA, and centrifuged at 10000 rpm for 10 min. The protein pellets were washed sequentially 2× with 10% TCA, 2× with ethanol and once with diethyl ether. The samples were then dried under vacuum and resuspended in electrophoresis sample buffer containing 0.01% sodium phosphate (pH 7.2), 1% sodium dodecyl sulphate (SDS), 6 M urea, 1% β-mercaptoethanol, 0.01% bromophenol blue. Solubilization of the sample pellets required 20–30 min of incubation at 37° C and moderate mixing. The samples were applied to a 7.5–15% linear polyacrylamide gradient gel, overlaid by a 5% stacking gel, for SDS-denaturing TRIS/glycine/chloride discontinuous electrophoresis as described (Ausubel et al. 1987). The gel was dried and subjected to autoradiography to permit identification and alignment of the *10B* and *10A* proteins before excision and quantitation of incorporated radioactivity. Protein bands corresponding to *10B* and *10A* were cut out from the gel and rehydrated with 100 μl H_2O for 30 min; 10 ml of Fluorosol, a one-step scintillator/protein digestant (National Diagnostics), was added. The samples were kept shaking for 2 to 3 days at 37° C to elute all the radioactivity from the gel slices, as monitored by periodic counting of the samples. Quantitation of the ratio of radioactivity found in the *10B* and *10A* bands for each lane of the gel was calculated as follows: [cpm in *10B*/(cpm in *10B*+cpm in *10A*)] × 100.

Results

Attempts to perturb the gene 10 *frameshift using different ribosomal backgrounds*

Because T7 gene *10B* protein arises by an apparent translational frameshift in the reading of the gene *10A* mRNA (Dunn and Studier 1983; Sipley et al. 1991; Condron et al. 1991), we sought to examine the effects of a range of host translational fidelity conditions on the frequency of the frameshift. We utilized a collection of ribosomal fidelity mutants in an F$^-$ isogenic background (T7 is a female-specific phage) in combination with streptomycin, an agent known to cause translational misreading. Frameshifting during the course of T7 infection was determined by quantitation of the ratio of gene *10* products expressed during intervals late in T7 infection as revealed by [^{35}S]methionine pulse-labeling and SDS-polyacrylamide gel electrophoresis (PAGE) analysis.

Figures 1 and 2 show time courses of the synthesis of T7 phage proteins during infection under some of the different conditions of translational accuracy that were tested. During infection, T7 gene expression is controlled through ordered and sequential transcription. Three classes of proteins are made; Class I proteins arise from host transcription and include: T7 RNA polymerase, enzymes that inactivate host enzymes, T7 DNA ligase, and other proteins of unknown function. Class II proteins arise from T7 RNA polymerase transcription and encode enzymes required for DNA metabolism, and inactivation of the host RNA polymerase. Class III proteins encode mostly phage structural proteins of which gene *10* is the most abundantly expressed (Dunn and Studier 1983).

Figure 1A presents the phage-specific proteins (mostly class III, some class II) produced late in infection of cells containing wt ribosomes, "hyperaccurate" streptomycin-pseudodependent ribosomes without streptomycin (Sm^p without Str), and "inaccurate" ram ribosomes. The outer lane (gene *10 amber*), which lacks the gene *10A* and *10B* proteins, serves as an aid to help identify the individual *10A* and *B* bands in the other lanes (the band immediately above *10B* is a host band which is labeled due to incomplete shutoff of host protein synthesis; see Fig. 2). As indicated, the gene *9* protein is the major band migrating between the *10A* and *10B* proteins under these conditions of electrophoresis. In an effort to facilitate quantitation of *10B* relative to *10A*, a T7 gene *9 amber* mutant was utilized in these experiments to minimize incorporation of label into protein migrating in the *10A* region of the gel. However, the same results were also obtained with wild-type T7 (data not shown). Overall the amount and kinetics of synthesis of the late T7 proteins are similar in the wt and ram strains; the levels of *10B* and *10A* expression also appear proportional. However in the Sm^p strain, total incorporation of label is noticeably reduced, and synthesis of T7 late proteins appears delayed; in some experiments, proteins characteristic of earlier timepoints are still apparent at later timepoints (e.g., gene *2.5* in Fig. 2). Quantitation of the frameshift ratio expressed as [*10B*/(*10B*+*10A*)] × 100, of each lane of the gel is presented in Fig. 1B. No significant difference in the amount of frameshifting among the three strains was apparent.

Figure 2 shows the complete time course of infection of the Sm^p strain with and without streptomycin. Strikingly different kinetics of infection as well as total incor-

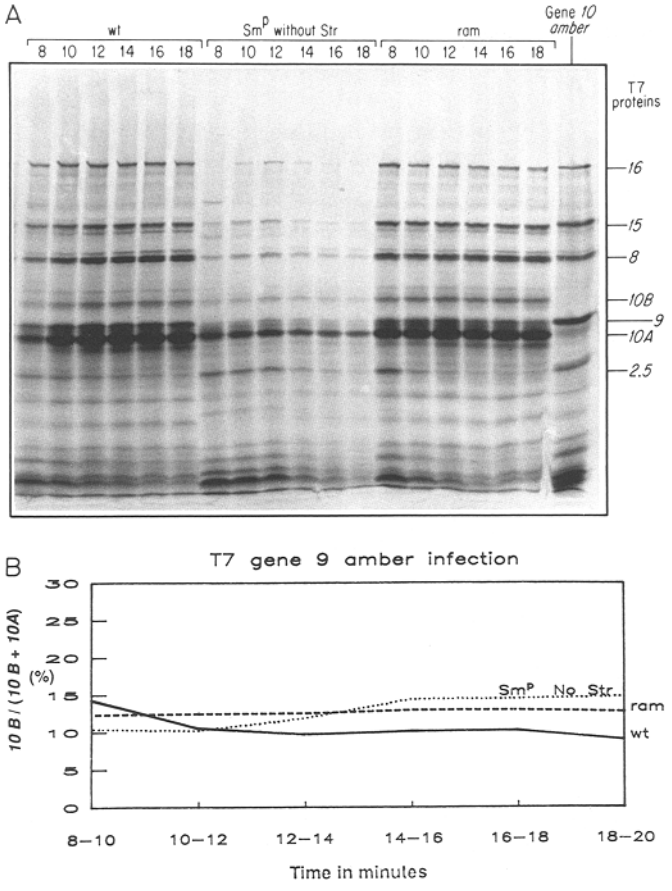

Fig. 1A and B. Time course of late protein synthesis during T7 gene *9* amber phage infection of cells with wt ribosomes (wt), streptomycin-pseudodependent ribosomes without streptomycin (Sm^P without Str), and ram ribosomes (ram). **A** Cultures were each infected with T7 *9am17*; after 8 min, the cultures were pulse-labeled with [^{35}S]methionine for 2 min intervals, extracted, and analysed by electrophoresis as described in the Materials and methods. Lane numbering indicate the time of infection in minutes. The phage amber mutant T7 *9am17* appeared to be somewhat leaky in these experiments. T7 gene *10* amber phage (T7 *10am13*) were used to infect wt strain XAc as above, and the protein products were run in the outside lane of the gel to serve as an internal control for the precise location of the gene *10A* and *10B* proteins. Quantitation of the frameshift ratio. The range of actual counts/min recovered from the gel slices varied from approximately 50000 to 384000 for *10A*, and 9000 to 55000 for *10B*

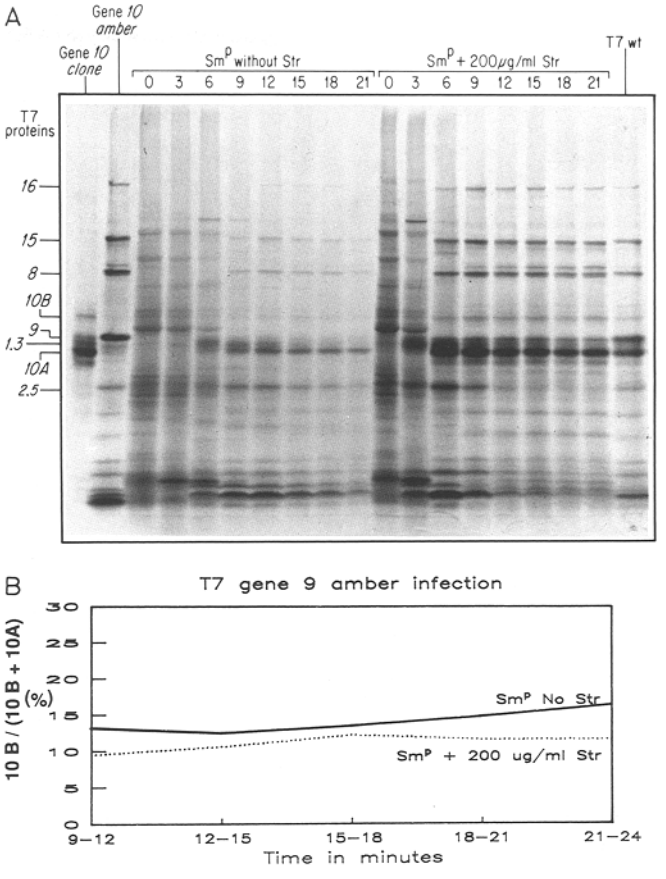

Fig. 2A and B. Time course of synthesis of T7 gene *9* amber phage proteins during infection of streptomycin-pseudodependent cells without streptomycin (Sm^P without Str) and streptomycin-pseudodependent cells with streptomycin (Sm^P+200 μg/ml Str). **A** Cells were grown as described in the Materials and methods and subsequently treated essentially as in Fig. 1; however, 3-min pulse labels were utilized to show the proteins made from the onset of infection as well as the late proteins. Lane numberings indicate the time of infection in minutes. The lane marked "Gene *10* clone" was from BL21 (DE3)/pAR3625, pLysS cells induced with 0.4 mM isopropylthiogalactoside (IPTG) for 30 min and labeled with [^{35}S]methionine as in Fig. 1. **B** Quantitation of the frameshift ratio; the range of actual counts/min recovered varied from approximately 26000 to 311000 for *10A* and 5000 to 33000 for *10B*

poration of label are noticeable between the two series (e.g., compare lanes 6 and 9 of "Sm^P without str" with lanes 6 and 9 of "Sm^P+200 μg/ml Str"). However, despite these striking differences, no significant difference in gene *10* frameshifting was detected with the addition of streptomycin (Fig. 2B).

In another attempt to assess effects of translational fidelity perturbations on the gene *10* frameshift, we examined the courses of late T7 infection of the host strain with wt ribosomes, and with wt ribosomes grown for several generations in 1.0 μg/ml streptomycin (slightly less than 50% growth rate inhibition) and 1.5 μg/ml streptomycin (greater than 50% growth rate inhibition). Wild-type levels of T7 late proteins were apparent throughout the timepoints at both streptomycin concentrations (data not shown), and there were no apparent differences in the levels of *10B* or *10A* protein production, as confirmed by quantitation of the frameshift (not shown).

Effects of the different ribosomal backgrounds on phage production and DNA synthesis

T7 wt phage morphogenesis was monitored in a single-step growth experiment concurrent with the analysis of T7 late proteins expressed under the different conditions of altered translational fidelity. The data shown in Fig. 3 were obtained under the same conditions as those in Figs. 1 and 2, except that wild-type phage were used. The half-lysis times during the T7 single-step growth

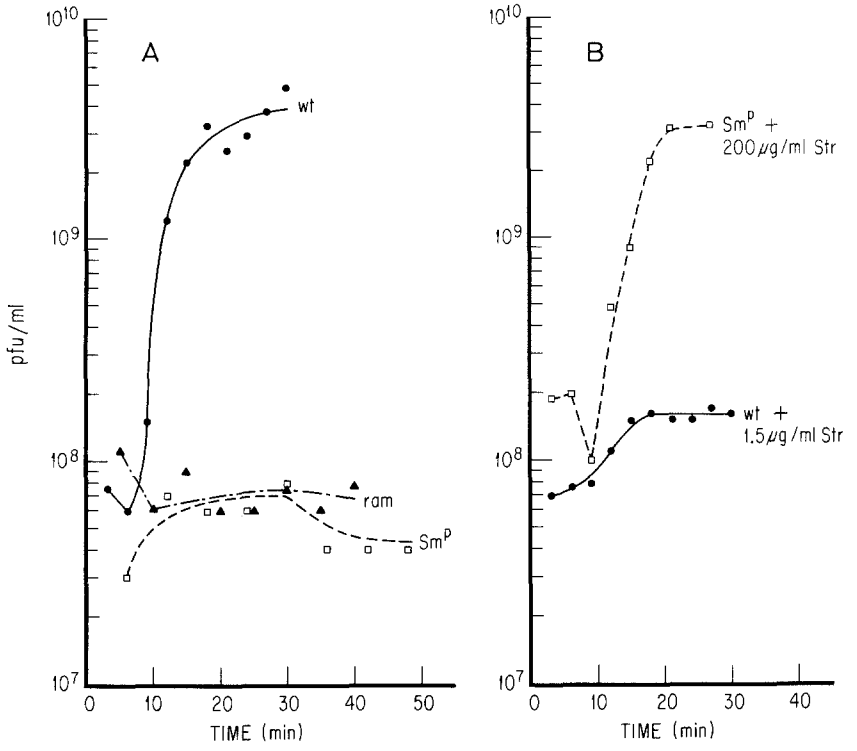

Fig. 3A and B. T7 single-step growth experiments under different ribosomal fidelity conditions. T7 wt phage morphogenesis was monitored concurrently with SDS-PAGE analyses of different translational fidelity conditions similar to those presented in Figs. 1 and 2. Cultures were infected with wt T7 and periodic samples of the infected cultures were removed and treated with $CHCl_3$, lysozyme (50 μg/ml), and 10 mM EDTA. The samples were centrifuged at 10000 rpm for 2 min and serial dilutions of the supernatant were assayed for plaque-forming units on a lawn of XAc (wt ribosomes). Each infected culture was also monitored for "half-lysis time," which is defined as the time in minutes for the turbidity (measured in a Klett-Summerson colorimeter) to decrease by half following addition of phage to the culture. Half-lysis times obtained for each condition were: wt, 17 min; wt+1.5 μg/ml Str, 27 min; ram, 25 min; Sm^P, 43 min; Sm^P+200 μg/ml Str, 17 min

Table 2. Production of T7 particles in infected wt, ram, and Sm^P cultures

Strain	Percent Survivors	CsCl band (A_{260}/ml)
Sm^P, no Str	100	0.204
ram	89	0.447
wt	3	1.836

Cells were grown at 37° C in M9 to 2×10^8/ml and infected with T7 at a multiplicity of 5. After 40 min, the cultures were treated with $CHCl_3$, and centrifuged. These lysates were used to infect a standard wt strain (XAc) by adding 0.5 ml of lysate to 2 ml of culture. Five minutes later, portions of the culture were subjected to serial dilutions on ice, and the titres of the cells determined on agar plates. The percent survivors refers to the cell titre obtained after adding the indicated lysate, divided by the titre of parallel uninfected cells, $\times 100$.

In another experiment, 100 ml cultures of the three different strains were infected with T7 at a multiplicity of 3, and after 40 min the cultures were subjected to semi-purification procedures for T7 as described in the Materials and methods. These lysates were banded in a CsCl step-gradient, as described by Studier (1969). Fractions were collected from each sample, and the A_{260}/ml determined across the gradient. Data shown are the A_{260}/ml of the visible T7 band obtained in the wt lysate, and the comparable A_{260}/ml obtained in the parallel fractions of the gradients from the other two lysates

experiment are presented in the legend to Fig. 3. As shown in Fig. 3A, wt ribosomes yielded the most viable T7 progeny phage (with a burst size of ~20 phage per infected cell in this experiment) and these cultures lysed fastest. Addition of a sublethal amount of streptomycin (Fig. 3B) to the wt culture markedly inhibited the production of viable progeny phage (burst ≃1 phage per infected cell), despite no evident differences in a corresponding SDS-PAGE analysis (not shown).

Infection of the Sm^P strain in the absence of streptomycin was inhibited: few if any phage particles capable of killing a susceptible host were produced, and very little material from this infection banded in CsCl at the position of mature T7 phage particles (Table 2). These cultures also were the slowest to lyse. We were surprised that the infection was so inefficient because total protein synthesis, as monitored by [^{35}S]methionine incorporation, is only reduced about 50% in the absence of streptomycin (Figs. 1, 2), and T7 can even form plaques when this strain is used as indicator. The addition of streptomycin to the Sm^P strain increased the level of viable progeny phage by more than an order of magnitude (burst ≃15 phage per infected cell) and reduced cell lysis time (see legend to Fig. 3B). The apparent contradiction between the ability to form plaques on this strain and the reduced yield of particles in the single-step growth experiments may be a consequence of the different conditions for each kind of assay: in the plaque assay, serial dilutions of the phage stock lead to an initial multiplicity on the plate of less than 1×10^{-6}, whereas the single step growth experiments used a multiplicity of 3–5. Perhaps the Sm^P cells, already compromised for growth, are unable to sustain productive phage infection under the stress of the higher multiplicity of infection.

The ram mutant, which has kinetics of infection and protein synthesis similar to those of cells with wt ribosomes, also produced few viable progeny phage (~1 phage per infected cell) (Fig. 3A; Table 2). In other experiments where T7 had a larger burst size on wt cells (up to 100 phage per infected cell) compared to the experiments of Fig. 3, the yield of viable progeny phage

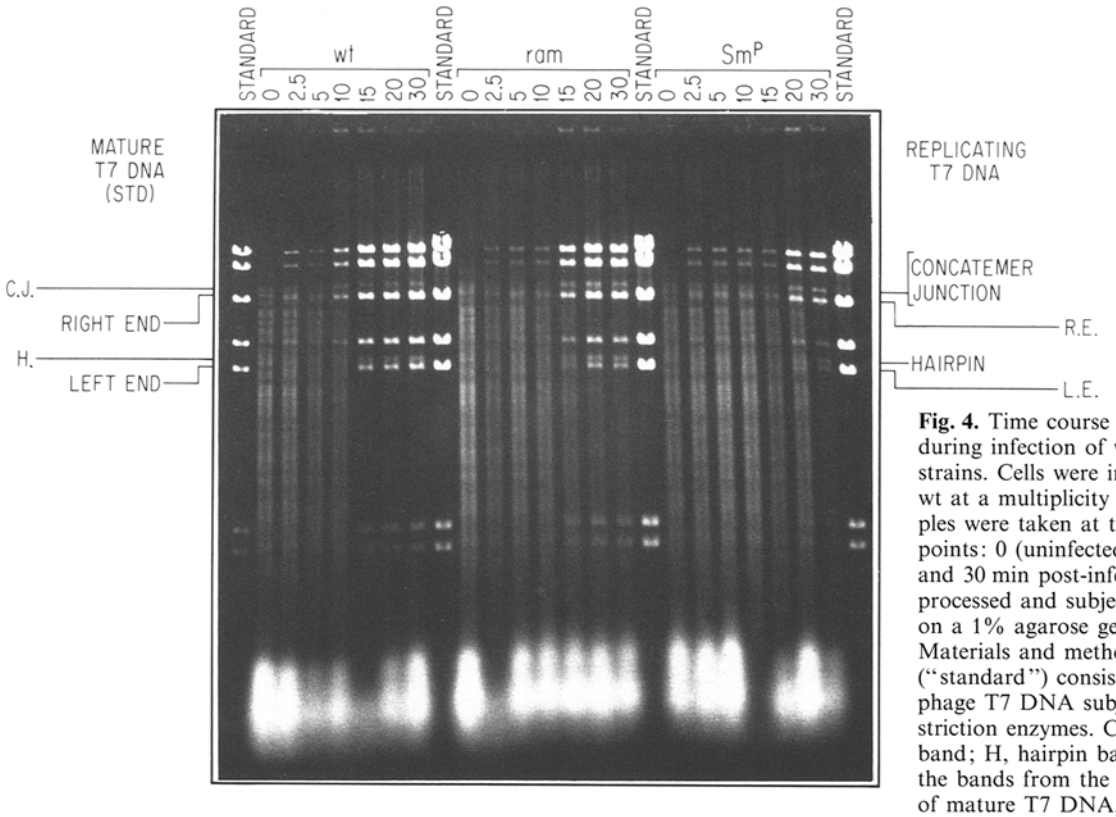

Fig. 4. Time course of T7 DNA synthesis during infection of wt, ram, and Sm^p strains. Cells were infected with phage T7 wt at a multiplicity of 5 at 37° C, and samples were taken at the following time points: 0 (uninfected), 2.5, 5, 10, 15, 20, and 30 min post-infection. Samples were processed and subjected to electrophoresis on a 1% agarose gel as described in the Materials and methods. Marker lanes ("standard") consisted of 300 ng mature phage T7 DNA subjected to the same restriction enzymes. CJ, concatemer junction band; H, hairpin band; R.E. and L.E. are the bands from the right end and left end of mature T7 DNA, respectively

from the ram mutant was still at least 20-fold below that seen with wt cells. Despite the reduced burst size after infection of the ram strain, T7 is nevertheless able to form plaques on this strain.

Since the effects of these different ribosomal backgrounds on the extent of T7 protein synthesis did not seem sufficient to explain the inefficient infections, we also examined T7 DNA synthesis in these strains. As T7 replicates it forms concatemers, i.e., long molecules containing tandemly repeated T7 genomes joined in a head to tail arrangement through a shared copy of a 160 bp long terminal repeat. The concatemer is thought to provide an intermediate through which the 3′ ends of the T7 DNA can be replicated. The concatemers are then processed into unit length molecules, and the terminal repeat is duplicated, as the DNA is packaged into virions in a reaction that is closely linked to phage morphogenesis (Studier and Dunn 1983).

Figure 4 presents the electrophoretic analysis in a 1% agarose gel of a restriction endonuclease digest of total DNA isolated during the course of T7 infection of strains with wt, ram, or Sm^p ribosomes. The mixture of enzymes used to digest the DNA yields a characteristic pattern of bands from mature T7 DNA and two additional bands from replicating T7 DNA. Particularly notable is the presence in the digests from infected cells of bands from the right and left ends of the mature DNA, which only arise by processing of the concatemer junction. The patterns show clearly that T7 DNA is replicated in both the ram and Sm^p strains; however, the Sm^p strain shows considerable delay and reduction in the amount of T7 DNA synthesized compared to the wt strain, while the ram strain shows only a slight delay in T7 DNA synthesis. The patterns also indicate that the replicated DNA in the ram and Sm^p strains can be packaged as judged by the appearance of mature right- and left-hand end fragments. These results suggest that the reduced progeny phage yield in the ram strain is not caused by a failure to replicate and package T7 DNA. The reduced amount of replicating DNA in the Sm^p strain, however, is probably a major contributing factor to the inefficient infection in this strain.

Comparable results were obtained when single-step growth experiments were performed, similar to those described in Fig. 3, using the closely related bacteriophage T3 (data not shown). However, T3 infection of cells containing wt ribosomes (burst size = 50–100 phage per infected cell) does not seem to be as sensitive as T7 to a sublethal amount of streptomycin (1.5 μg/ml, burst ≃40 T3 phage per infected cell; and 2.0 μg/ml, burst = 10 T3 phage per infected cell). To assess possible translational effects on phage growth in general with these strains, we carried out single-step growth experiments using T4, a lytic phage unrelated to T7 and T3 (data not shown). The translational effects on progeny phage yield are qualitatively similar to those seen in T7 and T3 infection; however, quantitatively the effects are less significant. The wt strain and streptomycin-pseudodependent strain with 200 μg/ml streptomycin produced a similar burst size of 100 pfu per infected cell. When the streptomycin-pseudodependent strain was grown without streptomycin, the phage yield dropped approximately 2.5-fold (~40 pfu per infected cell). Production of viable progeny phage during T4 infection was most

severely reduced in the ram strain which had a burst of about 10 pfu per infected cell, which is approximately 10-fold less than that obtained from cells with wt ribosomes.

Discussion

We have found no effect of changing ribosomal fidelity on the frequency of the phage T7 gene *10* frameshift. Very similar ratios of 10B to 10A protein were observed in each condition we tested. We would not have expected a complete abolition of frameshifting in the restrictive *rpsL* mutant due to the fact that certain essential host genes require programmed frameshifts to generate their products; however, at least some reduction in the frequency of frameshifting might have been anticipated, particularly given the fact that these cells are already seriously compromised for growth. The lack of effect of ribosomal fidelity on the high-frequency gene *10* frameshift contrasts with earlier studies on low-frequency shifts (Atkins et al. 1972; Weiss and Gallant 1983). The fidelity alleles would seem to affect only the very low-frequency errors in maintenance of the reading frame; on the other hand, the fidelity conditions tested here do not have significant impact on an apparent programmed shift. Similar to our findings, Tsuchihashi (1991) was unable to alter the high-frequency *dnaX* frameshift by increased ribosomal fidelity. Other workers have also suggested that frameshifting was not affected in streptomycin resistant (Sm^r) strains. Parker and Precup (1986) reported the lack of effect of a restrictive *rpsL* (*StrA1*) allele on putative frameshifting in phage MS2, and Spanjaard and van Duin (1988) found that high level +1 frameshifting occurs during translation of an MS2 coat protein/rat interferon protein gene fusion in an Sm^r host. However, in the latter example, it is not clear whether the authors used a restrictive allele of Sm^r.

Since the *10A* to *10B* frameshift occurs under all conditions we have tested, it is apparently an intrinsic property of the primary sequence of the mRNA, and is not subject to controls of fidelity of translation, at least with respect to the fidelity variations tested in this work. Also, in vitro translation of gene *10* message in an *E. coli* extract results in production of both 10A and 10B even when high concentrations of ppGpp are added to the extract (J. Dunn, unpublished experiments), despite the expected increased translational fidelity in the presence of ppGpp (Gallant 1979; O'Farrell 1978; Wagner et al. 1982).

Other elements have been identified as controls in high-frequency frameshifting (reviewed in Atkins et al. 1990). The most general is a requirement for runs of several identical nucleotides that call for specific "shifty" tRNAs, which are capable either of pairing in other reading frames, or non-triplet pairing (as reviewed by Parker 1989). The amino acid sequence across the gene *10* frameshift site has been determined (Condron et al., 1991), and it appears that a shifty Phe-tRNA is involved that shifts at a run of four uracil residues. A second element is one that facilitates translational pausing in the region of the string of nucleotides, allowing the shift occur. This element may consist of combinations of the following: a termination codon (Craigen and Caskey 1986; Curran and Yarus 1988; Weiss et al. 1987, 1988), a stem loop structure (Jacks et al. 1988; Brierly et al. 1989; Flower and McHenry 1990; Blinkowa and Walker 1990; Tsuchihashi and Kornberg 1990; Bredenbeek et al. 1990; Dinman et al. 1991), a Shine-Dalgarno sequence (Weiss et al. 1988), or a "hungry" codon specifying a "rare" tRNA (Spanjaard and van Duin 1988; Spanjaard et al. 1990; Belcourt and Farabaugh 1990; Xu and Boeke 1990). An additional factor has recently been identified by Belcourt and Farabaugh (1990), who found that frameshifting in the yeast Ty retrotransposon is inhibited if the frameshift site is located too close to the initiation codon. It is not yet known if any additional element (besides the run of uracil residues) is involved in the gene *10* frameshift.

We were surprised to find rather severe effects of altered fidelity on T7 and T3 yield in single-step growth experiments. Examination of the T7 late phage proteins by SDS-PAGE revealed little if any difference between wt and ram strains despite a reduction in yield in the ram strain of viable progeny phage as well as particles capable of killing susceptible cells or banding in CsCl. Similarly, addition of a sublethal amount of streptomycin to cells containing wt ribosomes showed no obvious difference in synthesis of late phage proteins; however, production of viable progeny phage was again greatly reduced. Perhaps this is the result of mistranslation introducing missense amino acid substitutions that cannot be detected by SDS-PAGE; the same explanation could also account for inhibition of growth in the ram strain (see Gorini 1974).

The inefficient infection of the streptomycin-pseudodependent strain, unlike the inefficient infection of the ram strain (and possibly wt ribosomes plus sublethal streptomycin), was accompanied by moderately slower kinetics and reduced rates of synthesis of T7 late proteins; these effects were reversible by addition of streptomycin to the medium. The changes, however, did not seem substantial enough to account for the reduction in viable phage yield. Most likely a major constraint on production of T7 phage in the streptomycin-pseudodependent strain involves the observed decrease in the rate of T7 DNA synthesis. Whether the slower kinetics of infection in the Sm^p strain play a role in the reduction of yield from these infections is not clear; presumably this is a direct result of the property that Sm^p ribosomes in the absence of streptomycin are slower in translation (Ruusala et al. 1984). Perhaps slower translation leads to limitation for a specific gene product. If the product were vital in T7 or T3 infection for development of progeny phage, it would most likely be required in stoichiometric amounts and any reduction below a certain threshold level would hinder the infection. Alternatively, T7 and T3 may require the translational "leakiness" afforded by wt ribosomes, and it is possible that hyperaccuracy actually prevents flexibility needed for normal morphogenesis.

These issues are apparently not relevant during T4 infection; the decreased phage yields obtained during T4 infection of the Sm^p strain without streptomycin appear to be a direct reflection of the diminished capacity of the translational apparatus in the absence of streptomycin (Ruusala et al. 1984; Bohman et al. 1984; Andersson et al. 1986). Since some viable progeny phage are produced ($\sim$10-fold less than with wt ribosomes) during T4 infection of the ram strain, one might speculate that this is a consequence of the intrinsic translational inaccuracy of the ram strain but that not all errors in protein synthesis will be deleterious.

Restrictive effects of streptomycin-resistant strains on growth of MS2 (Chakrabarti and Gorini 1975), Qβ, λ, and filamentous phages (f1, fd, and M13) (Engelberg-Kulka et al. 1977, 1979) have previously been described. To the best of our knowledge, restrictive translational effects on T7 and T3 growth have not previously been noted in streptomycin-pseudodependent or ram strains. By contrast, some streptomycin-resistant male strains have been reported to grow T7 phage much better than wild-type males (Chakrabarti and Gorini 1975), with the increased translational accuracy apparently overcoming the F-mediated block to T7 morphogenesis (Kruger and Bickle 1987); this has been attributed to reduced *pif* gene expression (Molineux et al. 1989). We have also confirmed that a male version of the Sm^p strain used in this study plaques T7 many orders of magnitude better than the isogenic wt male strain, though still not as well as an F^- strain. Furthermore, addition of streptomycin to the Sm^p male somewhat decreased its ability to plaque T7 (data not shown). The male version of the Sm^p strain was also unable to plaque the male-specific phage MS2, thus making it analogous to the Class IV Sm^r strains as described by Chakrabarti and Gorini (1975).

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