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Permeability Lesions in Male *Escherichia coli* Infected with Bacteriophage T7

(nucleotide pools/amino acid transport/translational control)

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ABSTRACT **The abortive development of bacteriophage T7 in *E. coli* cells carrying F factors has previously been attributed to a lack of virus-directed modification of ribosomes in such cells. We find it unnecessary to postulate such translational control to explain the failure of T7 development. Instead, there is a general cessation of macromolecular syntheses around 8 min after T7 infection of F′ cells. This cessation is correlated with a sudden outflow of the entire acid-soluble pool of phosphorus-containing compounds and loss of the ability to accumulate amino acids. Manifestation of these defects requires expression of at least one T7 gene and one episomal gene.**

The development of bacteriophage T7 is abortive in *Escherichia coli* strains that harbor F factors (1). T7 kills F^+ or F′ hosts without inducing the chromosomal breakdown, viral DNA synthesis, or lysis characteristic of infection of F^- cells (2). Malamy and coworkers have suggested that the aborted development of T7 in male cells is due to specific discrimination against translation of "late" T7 messages (2–4). This suggestion is based on the following observations: the synthesis of "early" T7 proteins is similar in F′ and F^- cells, but the synthesis of "late" proteins (DNA polymerase, lysozyme, and major capsid protein) is virtually absent in F′ cells (2, 3). On the other hand, RNA extracted from infected F′ and F^- cells was concluded to be capable of directing the synthesis of T7 lysozyme and major capsid protein *in vitro* at roughly comparable levels (4). Translational control was attributed to the ribosome: translation of late T7 messages was said to require modification of ribosomes, which is blocked in F′ cells (2).

The abortive development of T7 in F′ cells does not appear to be due to such specific translation defects. Condit and Steitz have shown that translational machinery taken from T7-infected F′ cells is as competent for late T7 protein synthesis *in vitro* as the machinery from F^- cells (5). Moreover, *all* T7 macromolecular synthesis ceases abruptly 7–8 min after infection of F′ cells (5, 6). Here, we show that F′ cells become leaky 7–8 min after T7 infection, losing all the components of the pool of acid-soluble, phosphorus-containing compounds. Leakiness requires the function of at least one gene in the F episome and one in T7. This permeability change in T7-infected F′ cells, observed independently by Condit (7), would account for the cessation of macromolecular synthesis and the premature termination of T7 development. Since the leak develops after late T7 RNA transcription has begun but prior to late protein synthesis, the general cessation of macromolecular synthesis could be misinterpreted as evidence for translational control.

MATERIALS AND METHODS

Bacteria and Phage. *E. coli* FRAG(*thi*$^-$ *rha*$^-$ *gal*$^-$ *lac* Z_{x82}) and its F′*lac* derivative were obtained from Dr. W. Epstein, who derived them from *E. coli* K12 200PS; strain 371 ∇ lac-pro *str*^{r} was obtained from Dr. D. Morse. T7 wild type and the following mutants were provided by Dr. F. W. Studier: *am*23(gene 1), *am*29(gene 3)*am*28(gene 5)*am*147-(gene 6), deletion H1-*am*27(gene 1), deletion H3-*am*342(gene 1), deletion D111, and deletion LG37. Phage stocks were prepared by polyethylene glycol precipitation from lysates (8). The transmission coefficient of wild-type T7 on FRAG F′*lac* is less than 0.1 relative to FRAG F^-.

Chemicals. $^{35}SO_4^{-2}$ was purchased from ICN, $^{32}PO_4^{-3}$ from Schwarz/Mann, and L-[*U*-^{14}C]glutamine from New England Nuclear Corp.

Growth of Cells and Phage Infection. Bacteria were grown from a 1:100 dilution of a fresh overnight culture in M9 medium (9) supplemented with 0.1% casamino acids and 1 μg/ml of thiamine at 30°. For experiments with $^{35}SO_4^{-2}$ labeling, the casamino acids were omitted, $MgCl_2$ was present at 1 mM, and $MgSO_4$ at 0.05 mM. When the culture reached an absorbance of 0.6 at 650 nm (3×10^8 cells per ml), phage were added at a multiplicity of 10.

Polyacrylamide Gel Electrophoresis. Aliquots of infected cultures were boiled in the sample buffer of Laemmli (10) and electrophoresed on 20% acrylamide gels [acrylamide:bisacrylamide ratio 300:1 (11)] containing 0.1% sodium dodecyl sulfate. The dried gel slabs were autoradiographed using Kodak No-screen x-ray film.

Measurement of Nucleotide Pools. Cells were grown for three generations before infection in the presence of 150 μCi/ml of $^{32}PO_4^{-3}$. Before and at various times after infection with T7 *am*29*am*28*am*147$^-$, 1.0-ml aliquots were collected on 0.45-μm nitrocellulose filters (Millipore) and the filters were washed with 1.0 ml of M9. The combined wash and filtrate were collected directly into 0.1 ml of 88% formic acid. The filters were placed in vials containing 2 ml of 1 M formic acid and shaken in the cold for 15 min. Aliquots of these cellular extracts (0.2 ml) were applied directly to PEI-cellulose thin-layer plates (Brinkmann Instruments). Nucleotides were adsorbed from the filtrates by the addition of Norit to 2%. The Norit pellet was washed once with 1 mM HCl, once with distilled water, and was then eluted with 2 ml of 1 M ammonium hydroxide in 50% ethanol. Aliquots of the eluate (0.2 ml) were applied to the thin-layer plates. Chromatographic procedures were those of Cashel *et al.* (12) and Randerath and Randerath (13). Dried plates were autoradiographed with Kodak No-screen x-ray film.

Glutamine Accumulation Assay. Eight minutes after T7 infection of exponentially growing cultures, chloramphenicol

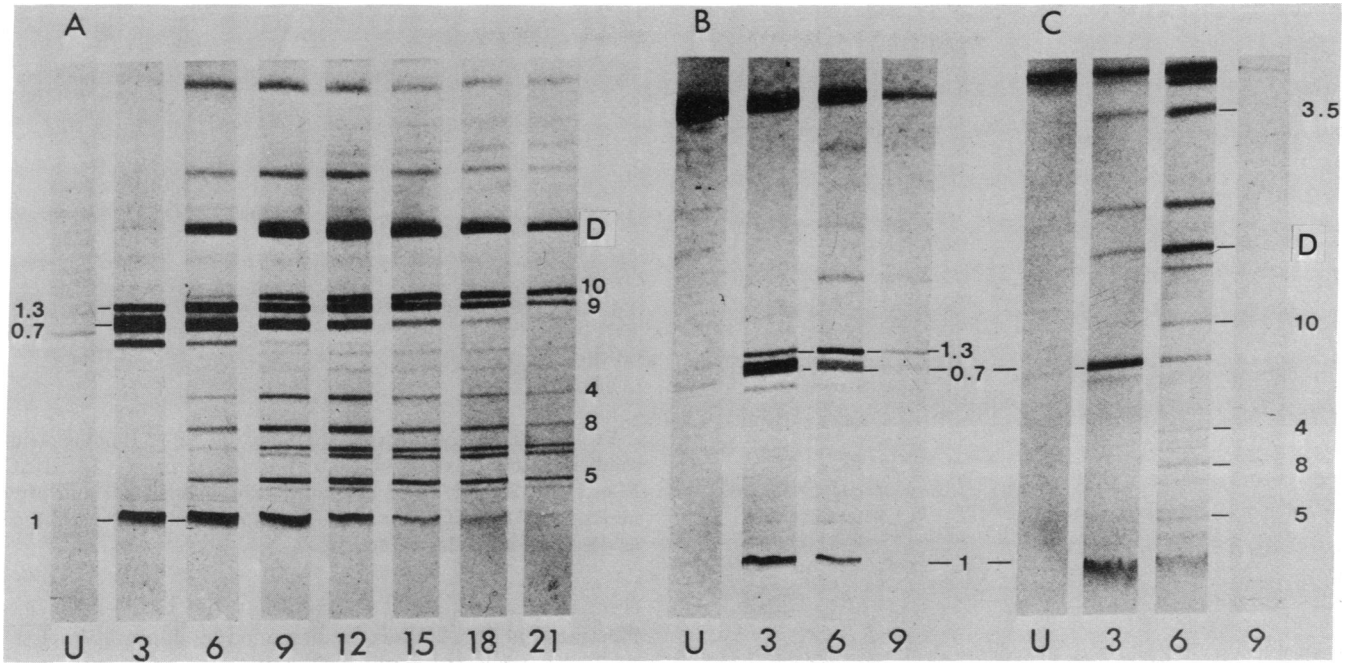

FIG. 1. Proteins synthesized after T7 wild type and LG37 infection of FRAG strains. Cells grown in low sulfate medium were UV-irradiated (6000 ergs/mm²) before infection. At the indicated times after infection, 0.5-ml samples were labeled with $^{35}SO_4^{-2}$ at 200 μCi/ml for 2 min, followed by a 3.5-min chase with 0.4% casaminoacids. After the chase, samples were boiled for 2 min in the denaturing buffer of Laemmli (10). The sample marked U is from uninfected cells. Equal sample volumes were applied to each slot. The electrophoresis origin is at the bottom. After electrophoresis, gels were dried and autoradiographed, as described in *Materials and Methods*, for 3 weeks. Numbers correspond to T7 gene products identified by Studier (15). The band labeled D is the T7 DNA-binding protein (F. W. Studier, personal communication) described by Reuben and Gefter (29). The gene 0.7 band is an unresolved precursor-product doublet (6). Note the absence of gene 1.3 product in LG37 infection. (A) T7 wild type, FRAG F⁻; (B) T7 wild type, FRAG F′*lac*; (C) T7 LG37, FRAG F′*lac*.

was added to a final concentration of 200 μg/ml. One minute later a 2.0-ml aliquot was transferred to a tube containing L-[*U*-^{14}C]glutamine at a final concentration of 0.5 μCi (0.1 μmol)/ml. Beginning 30 sec thereafter, 0.4-ml samples were removed at 1-min intervals and diluted into 4 ml of media. Samples were collected on 0.45-μm nitrocellulose filters, washed with 8 ml of medium, and dried. Radioactivity was determined in a toluene-based scintillator.

Isolation of an Episomal Mutant that Supports T7 Development. FRAG F′*lac* cultures were mutagenized according to the procedure of Adelberg *et al.* (14), with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine. Colonies surviving this treatment were replica-plated onto tryptone plates previously seeded with approximately 10^9 T7 phage. Colonies that failed to grow on the T7 plate were purified and tested for T7 plating efficiency and male characteristics. Of nine such isolates, one was chosen for further study. The episome from this strain was transferred into strain 371 and back to the FRAG F⁻ recipient. The final strain, designated *fex*⁻, plates T7 with an efficiency of nearly 1, plates MS2, and is able to donate the F′*lac* to suitable recipients. Similar mutants have been isolated from F′ strains by Morrison and Malamy (2).

RESULTS

Male E. coli Can Synthesize Late T7 Proteins. The patterns of protein synthesis during wild-type T7 infection of FRAG F⁻ and F′ cells are compared in Fig. 1A and B. In agreement with the results of others (2–4), we find that late T7 proteins are not made in the F′ cells. However, the defect in F′ cells does not appear to be restricted to late T7 proteins: comparison of the pulses beginning at 9 min in Fig. 1A and B shows that synthesis of the products of the early genes 1.0, 0.7, and 1.3 is also restricted in the F′ cells.

We are able to distinguish between specific discrimination against late T7 protein synthesis and a more generally widespread defect in T7 protein synthesis in F′ cells by using a T7 mutant called LG37 (15). This mutant carries a deletion removing part of gene 1.3 (T7 ligase) as well as the terminator of early T7 transcription (15, 16). As a consequence of the deletion, the entire T7 chromosome can be transcribed by *E. coli* RNA polymerase by "read-through" into the late region. In F′ cells, considerably more T7 RNA synthesis is resistant to inhibition by rifampicin in LG37 infection, as a consequence of read-through, than in wild-type T7 infection (17). The pattern of protein synthesis in LG37-infected F′ cells is shown in Fig. 1C. At least six T7 late proteins can be seen in the pulse from 6 to 8 min after infection. Their synthesis and that of the early proteins is shut off by 9 min after infection. We conclude from this experiment that the defect in T7-infected F′ cells is general and not specifically directed against late T7 protein synthesis.

Phosphorus Pools in T7-Infected Cells. Recent studies of nucleic acid and protein synthesis in T7-infected F′ cells reveal an abrupt cessation of all macromolecular metabolism occurring 7–8 min after infection (5, 6). These findings prompted an investigation into the nature of the nucleotide pools in such cells, since an adequate supply of the triphos-

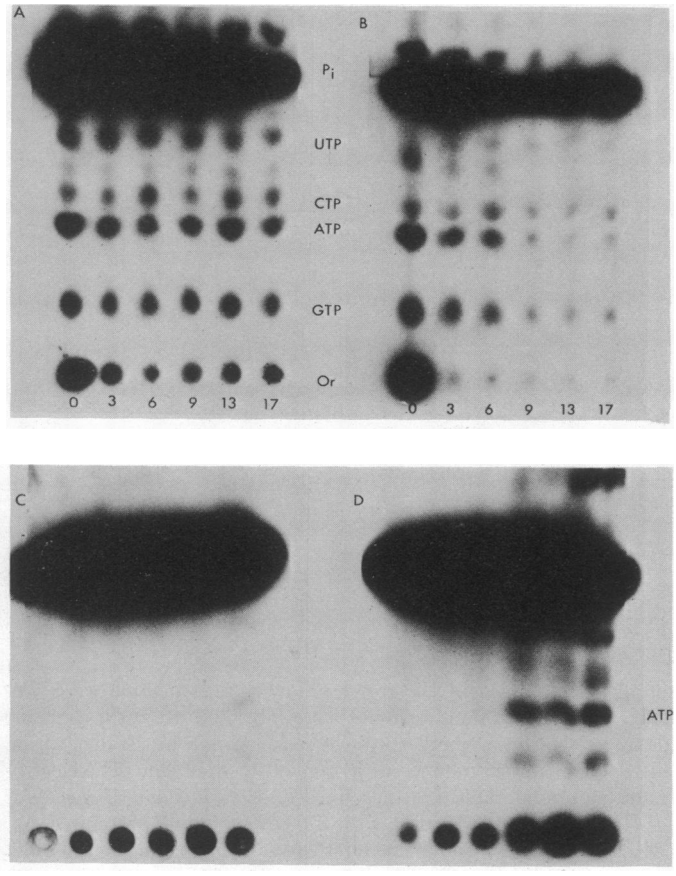

FIG. 2. Thin-layer chromatography of ^{32}P-containing acid-soluble material from T7-infected cells and culture media. Extracts were prepared at the indicated times after infection, chromatographed, and autoradiographed, as described in *Materials and Methods*, for 36 hr. Chromatography origin is at the bottom. Sample volume, exposure, and development time were identical for F^- and F'*lac* pairs. (A) F^- cellular extracts; (B) F'*lac* cellular extracts; (C) F^- culture filtrates; (D) F'*lac* culture filtrates. The spot between UTP and CTP is probably dCTP (12). Material scraped from the plates at positions corresponding to ATP and GTP contains the same amount of ^{32}P at all times in (A) but drops 4-fold between 6 and 9 min in (B). At least half the radioactivity missing from the GTP and ATP spots in (B) is recovered in those spots in (D).

phates is required for both nucleic acid and protein synthesis. In these experiments T7 carrying *amber* mutations in genes 3, 5, and 6, which include the nucleases responsible for host chromosomal breakdown, were used. Since such breakdown occurs in the female but not in the male host (2), use of this mutant avoids possible difficulties from contributions of DNA breakdown products to the cellular pools. FRAG F^- and F'*lac* cultures were labeled with $^{32}PO_4^{-3}$ and infected, and the intra- and extracellular pools of acid-soluble compounds were analyzed as described in *Materials and Methods*. Fig. 2A shows the pattern of nucleoside triphosphates extracted from F^- cells at various times after T7 infection, demonstrating that intracellular levels of the four major triphosphates are maintained as late as 17 min. In the F'*lac* host, however, there is a gradual decline of all four triphosphates after 6 min (Fig. 2B). This loss could result from either degradation within the cell or leakage into the medium. When the culture filtrates from the samples used in Fig. 2A and B were similarly

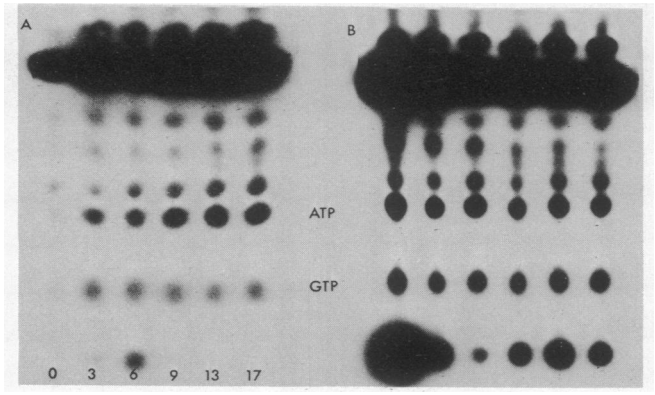

FIG. 3. Thin-layer chromatography of ^{32}P-containing acid-soluble material extracted from T7-infected cells. (A) FRAG F'*lac*, with 200 μg/ml of chloramphenicol added 2.5 min after infection; (B) FRAG F'*lac* fex^-. The experiments were performed as described in the legend for Fig. 2.

chromatographed, it was found that the intracellular loss illustrated in Fig. 2B was accompanied by appearance of the triphosphates in the medium (Fig. 2D).

The depletion of intracellular triphosphates in the F' cells is prevented by UV-irradiation of phage prior to infection (data not shown) or by the addition of chloramphenicol 2.5 min after infection (Fig. 3A). In addition, an episomal mutation, isolated by nitrosoguanidine mutagenesis of FRAG F'*lac*, which permits the development of T7 (designated *fex*$^-$ for *F-ex*clusion, see *Materials and Methods*), also prevents the depletion of intracellular triphosphates (Fig. 3B). Triphosphate loss is not prevented by UV-irradiation of bacteria prior to infection (data not shown).

Analysis of intracellular and culture filtrate material by the two-dimensional thin-layer technique of Randerath and Randerath (13) demonstrates further that by 13 min after infection not only triphosphates but all detectable nucleotide species, including possibly some nucleotide sugars, are lost from the F' cells and appear in the medium (Fig. 4). In the F^- host these species remain within the cell at this time. Some species (e.g., the monophosphates) appear more concentrated in the F'*lac* culture filtrate than in the F^- cellular samples, probably due to the Norit step used in the adsorption of nucleotides from the filtrate. Identical experiments performed without adsorption and elution of filtrate nucleotides from Norit showed similar distributions of these species in the F^- cells and F'*lac* filtrate (data not shown).

Chromatography of 13-min F'*lac* culture filtrate material in ammonium borate/acetate/KH_2PO_4 buffer systems (12), which resolve ribo- from deoxyribonucleoside triphosphates, has demonstrated that the majority of the triphosphate material remains in the ribo- form. The same is true of the intracellular pool in the F^- cells at this time (17).

Amino Acid Accumulation in T7-Infected Cells. The transport of glutamine in *E. coli* is markedly dependent upon cellular ATP levels (18). The depletion of nucleotide pools in T7-infected FRAG F'*lac* appears to coincide with the inability of these cells to accumulate glutamine; by contrast, T7-infected FRAG F^- retain the capacity to accumulate this amino acid (Fig. 5). Like the phosphorus pool depletion, the inhibition of glutamine accumulation in the infected F' cells is prevented by chloramphenicol added 2.5 min after infec-

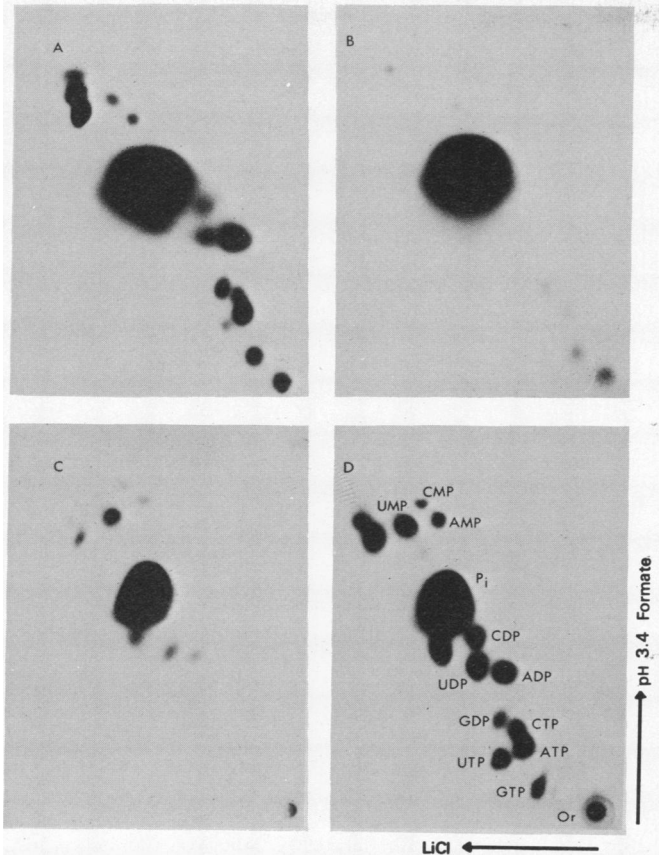

FIG. 4. Two-dimensional thin-layer chromatography of ^{32}P-containing acid-soluble material extracted from T7-infected cells and culture filtrates prepared 13 min after infection. Samples were prepared and chromatographed as described in *Materials and Methods*. (A) F^- cellular extract; (B) F′*lac* cellular extract; (C) F^- filtrate; (D) F′*lac* filtrate. The origin for the two-dimensional procedure (13) is at the lower right. Spots were identified by comparison with data in ref. 13. The spot just below P_i is probably UDP-glucuronic acid; the two to the left of UMP are also nucleotide sugars or, possibly, pyridine nucleotides (13).

tion and is not seen in F′ cells carrying the episomal *fex*$^-$ mutation (Fig. 5). The glutamine accumulation lesion may also be prevented by UV-irradiation of phage prior to infection (Fig. 5).

In an attempt to identify the T7 gene involved, a number of well-characterized T7 mutants were screened for possible inability to inhibit glutamine accumulation in F′ cells (Fig. 5). Most notably, phage carrying an *amber* mutation in gene 1, which codes for the RNA polymerase required for the expression of the entire late region of the T7 genome (19, 20), are still able to inhibit glutamine accumulation in FRAG F′*lac*. Since such mutants permit the synthesis of only the five early T7 proteins (15), one of these appeared to be required for inhibiting glutamine accumulation. However, T7 deletion mutants H1-*am*27, H3-*am*342, D111, and LG37, which as a group cover the entire early region, all inhibit glutamine accumulation (Fig. 5). Therefore, none of genes 0.3, 0.7, 1.1, or 1.3 is responsible for accumulation inhibition. A direct role for the gene 1 peptide may be ruled out since T7 *am*23, which carries an *amber* mutation at the amino-terminal end of gene 1, also inhibits glutamine accumulation (data not shown).

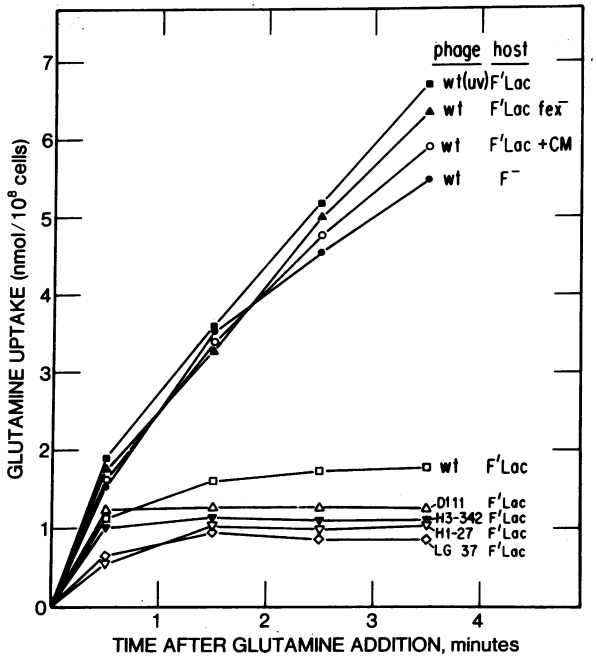

FIG. 5. Uptake of glutamine by T7-infected cells, measured as described in *Materials and Methods*. The phage strains used carry deletions of the following genes: H1-*am*27, part of genes 0.3 and 0.7; H3-*am*342, gene 0.7; LG37, genes 1.1 and 1.3; D111, gene 0.3 (ref. 16 and F. W. Studier, personal communication). Strains H1-*am*27 and H3-*am*342 carry gene 1.0 *amber* mutations *am*27 and *am*342, respectively. For exact map positions of deletions, see ref. 16. In the experiment with chloramphenicol (CM), the drug was added to 200 μg/ml at 2.5 min after infection. For the infection with UV-irradiated T7, phage were subjected to a dose of 6000 ergs/mm².

DISCUSSION

It no longer seems necessary to invoke selective translational controls to explain the restriction of T7 development in F′ cells. First, Condit and Steitz have shown that the translational machinery in such cells can translate late T7 messages *in vitro* (*5*). Second, we have shown that many late T7 proteins are made *in vivo* in the special case of LG37 infection of F′ cells. Finally, altered permeability of the F′ cells around 8 min after infection is sufficient to explain the apparent accumulation of *some* late T7 mRNA without late T7 protein synthesis.

The altered permeability of T7-infected F′ cells is manifested in a number of ways. Condit observed both increased accessibility to orthonitrophenyl-β-D-galactoside, the synthetic substrate for β-galactosidase normally excluded by *E. coli*, and a loss of ATP (7). We observed loss of all the acid-soluble phosphorus-containing compounds and inhibition of glutamine, proline (17), and K^+ accumulation (17). D. Rhoads has observed loss of intracellular K^+ (personal communication).

The permeability change resulting in nucleotide loss and inhibition of glutamine accumulation requires interaction of at least two genes, one on the episome and one on T7. Expression of one or both genes can be blocked by chloramphenicol added 2.5 min after infection. The episomal gene can be mutated to permit T7 development without affecting episomal replication or transfer, or pilus formation. Anthony *et al.* have shown that a mutant F plasmid that fails to restrict the growth of the T7-related phages T3, φII, and τ has a deletion

of the region extending from 33 kb to 43 kb on the standard F map (21). Presumably our *fex* mutation lies within this region. Morrison and Malamy have previously identified episomal mutations involved in T7 exclusion (2). They isolated 32 mutants, of which four had phenotypes similar to our *fex*$^-$ strain. The other 28 had a partially permissive phenotype: they restrict T7 development somewhat later than the wild-type episome and permit a very small burst of phage. Morrison and Malamy interpret these results to mean that two episomal genes must be mutated to provide the *fex*$^-$ phenotype. On the other hand, their observations are also consistent with two classes of mutant states of the same gene.

The T7 gene has not been identified yet. It does not appear to be one of the nonessential early genes, because deletion of each does not affect the permeability change. On the other hand, the function can be destroyed, in T7, by very high doses of UV. The UV target size for survival of this function in deletion H1 is roughly comparable to that of protein kinase (gene 0.7) in wild-type T7 (17). Following Brautigam and Sauerbier (22, 23), the radiation experiments suggest that gene 1 itself is the target, and that the T7 function responsible for the permeability change is a promoter-proximal late gene. If this is so, a very low level of gene 1 function, present in gene 1 *amber* infected cells, provides enough of the postulated late function to cause the membrane change. Since electrophoretic analysis of the T7 proteins synthesized in F′ cells (ref 2, and Fig. 1) shows only the known early proteins, the presumptive late protein responsible for the membrane change either has a very low molecular weight or is produced in minute amounts.

It is tempting to draw an analogy between the molecular events occurring in T7-infected F′ cells and those previously observed in cells treated with colicins K or E1 (24, 25). Both phenomena are associated with inhibition of macromolecular synthesis, cell permeability changes, and decreased intracellular ATP levels. There are, however, important differences between T7 infection and colicin treatment. Colicins E1 and K lower the intracellular level of ATP without causing ATP to leak into the medium (26). Moreover, the ATP destruction and inhibition of macromolecular synthesis which occur in colicin K treated cells are partially reversible by mutation of the gene (*uncA*) for the Ca^{++}–Mg^{++}-activated ATPase (27). This mutation does not overcome the inhibition of DNA and RNA synthesis and loss of nucleoside triphosphates in T7-infected F′ cells (Britton and Haselkorn, unpublished observations).

T7 infection of F′ cells appears to bear more resemblance to the abortive infection of *E. coli* Co2770 or *Shigella dysenteriae* Sh (P2) by T-even phages or T5 (28). Fields found that such infections are characterized by an arrest of RNA and protein synthesis, transport lesions, and leakage of ATP from the cells into the medium. The primary lesion in these cases is one of membrane permeability, attributed to an unknown mechanism involving the P2 prophage resident in *E. coli* Co270 and Sh (P2) (28). T7 infection of F′ cells may be more amenable to further analysis because a gene on the episome, mutated in our *fex*$^-$ strain, is known to be necessary for the membrane lesion.

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