*DNA TRANSFER FROM PHAGE T5 TO HOST CELLS: DEPENDENCE ON INTERCURRENT PROTEIN SYNTHESIS**

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Communicated by A. D. Hershey, March 11, 1965

Transfer of the phage T5 DNA molecule from an infecting phage particle to an *E. coli* cell can be divided experimentally into two steps: transfer of an initial portion (the first-step-transfer or *FST section*) amounting to about 8 per cent of the whole molecule, and transfer of the remainder. Complexes in which only the FST section has been transferred (*FST complexes*) lose their phage-producing ability upon blending. When transfer is allowed to go to completion under appropriate conditions, the complexes become *stabilized*, i.e., their phage-producing ability becomes resistant to blending.[1-3]

Unexpectedly, chloramphenicol was found to inhibit stabilization. Since, under the same conditions but without chloramphenicol, the second step of DNA transfer and stabilization were known to occur at a similar rate, it appeared that completion of DNA transfer might actually require the synthesis of one or more proteins not previously available in the host cell. If so, a likely candidate for inducing this synthesis was the FST DNA section.

This paper presents conclusive evidence that transfer of the FST section to the host cell occurs in the absence of protein synthesis and that transfer of the rest of the T5 DNA molecule requires fresh protein synthesis. The data are consistent with the proposal that the FST section itself contains the information for synthesis of the needed protein.

Materials and Methods.—T5 *st* and *E. coli* F[4, 5] were used in most of the experiments. An arginineless mutant of *E. coli* F was isolated for use in one kind of experiment.

The synthetic nutrient medium MGM and the buffer have already been described.[4] $CaCl_2$ ($10^{-3}M$) was present throughout the phage experiments.

Radioactive phage was prepared by infecting host cells grown in MGM supplemented with P^{32} as orthophosphate. The lysates were treated with pancreatic DNase, filtered through celite, and purified by differential centrifugation. The average labeling was 1–2 P^{32} atoms per phage.

The general experimental procedure is as follows. Cells are grown to about 2×10^8/ml in MGM, centrifuged, and resuspended in buffer at about 5×10^9/ml. After 5 min for equilibration to 37.5°, phage is added and incubation continued for 10 min, giving FST complexes. When chilled and blended, such complexes retain only about 10 per cent of the adsorbed phage DNA and are incapable of phage production. If unblended FST complexes are diluted to $1–2 \times 10^8$ cells/ml in MGM at 37.5° and incubated for 15 min, the rest of the phage DNA is transferred and the complexes become stabilized (blendor-resistant). For blending, chilled samples are treated for 6 min in a Servall Omni-mixer connected to a Variac set at 130 volts.

Stabilized complexes are assayed after blending and centrifuging (to discard unadsorbed phage) and are expressed as per cent of unblended controls. The unblended controls must be allowed to stabilize before centrifugation, since adsorbed

TABLE 1

IMPORTANCE OF PROTEIN SYNTHESIS FOR TRANSFER OF T5 DNA AND FOR STABILIZATION

Expt. no.	Treatment	Complexes Prepared under FST Conditions (5×10^9 cells/ml) Stabilized complexes, %	Complexes Prepared under FST Conditions (5×10^9 cells/ml) Transferred P^{32}, %	Same, after Dilution to 10^8 Cells/ml Stabilized complexes, %	Same, after Dilution to 10^8 Cells/ml Transferred P^{32}, %
1	No CM	1*	9	87	62
	CM	1	10	1	9
2	No arginine	1	10	3	11
	Arginine	—	—	98	66
3	No addition	1	10	89	59
	FPA	1	9	11	15
	FPA + PA	1	9	92	57

* The 1% surviving infective centers are probably due to adsorbed phages which survived shearing. These would make a small contribution to the "transferred P^{32}."

Experiment 1: Two samples of *E. coli* F were infected with P^{32}-T5. Chloramphenicol (CM, 50 μg/ml) was added to one sample 5 min before the phage and maintained throughout the incubation periods. Measurements were made (*a*) after incubation for 10 min at 5×10^9 cells/ml in buffer, and (*b*) after dilution to 10^8 cells/ml in MGM and incubation for 15 min.

Experiment 2: An argineless mutant of *E. coli* F was used. The cells were starved for 30 min in buffer at 37.5° with aeration. Aeration was stopped and P^{32}-T5 added. After incubation at 5×10^9 cells/ml, samples were diluted in MGM with or without arginine (20 μg/ml). Measurements were made as for experiment 1.

Experiment 3: Three samples of *E. coli* F were infected with P^{32}-T5. The supplements, DL-*p*-fluorophenylalanine (FPA, 2×10^{-3} *M*) and L-phenylalanine (PA, 1×10^{-3} *M*), were added 5 min before the phage and maintained throughout the incubation periods. Measurements were made as for experiment 1.

particles can be broken by centrifugation and resuspension. When desirable, the control values were confirmed by assaying the total number of infective centers and subtracting the unadsorbed phage.

Transferred P^{32} is the phage P^{32} retained by host cells after blending and washing, and is expressed as per cent of adsorbed P^{32}. The latter is measured in unblended, centrifuged FST complexes.

Modifications of the general procedure are described with the experiments. In all experiments the multiplicity of each phage input was about 5. The data presented are from an illustrative experiment of each type.

Results.—Role of protein synthesis in T5 DNA transfer and stabilization: Table 1, experiment 1, shows that, at 5×10^9 cells/ml in buffer (usual conditions for transfer of FST DNA only), transfer of FST DNA is not affected by chloramphenicol (CM). Exposure of the cells to CM for longer times before phage addition did not alter the results. After dilution to 10^8 cells/ml in MGM (conditions for full DNA transfer and stabilization), further DNA transfer and stabilization occur only in the absence of CM. Values of 60–70 per cent for transferred P^{32} represent maximal transfer, even under conditions of single infection.[1]

The requirement for protein synthesis was confirmed by infection of an argineless mutant of *E. coli* F in the absence of arginine and infection of the usual host in the presence of *p*-fluorophenylalanine (FPA) (Table 1, expts. 2 and 3). Addition of arginine to the argineless auxotroph permits maximal DNA transfer and stabilization, and phenylalanine (PA) prevents the inhibitory effect of FPA (Table 1). The inhibitory effects of CM and FPA are largely reversed by dilution of unblended complexes in media lacking these agents (results not shown).

DNA transfer and stabilization in the presence of presynthesized "transfer" protein: Although blended FST complexes, as such, are killed as colony-formers and produce no phage, about 50 per cent of these complexes can be superinfected productively under the present experimental conditions. This fact makes some interesting experiments possible. On the hypothesis that the transferred FST section

TABLE 2

T5 DNA TRANSFER AND STABILIZATION IN THE PRESENCE OR ABSENCE OF PRESYNTHESIZED "TRANSFER" PROTEIN

Expt. no.	Pretreatment	Stabilized complexes, %	Transferred P^{32}, %
1	A. FST; CM after 10 min at 37.5°	43	30
	B. FST; CM throughout	1	8
	C. No FST; CM after 10 min at 37.5°	1	9
2	A. FST; 5 min at 37.5°	43	23
	B. FST; 0°	6	14
	C. No FST; 5 min at 37.5°	3	13

Experiment 1, Culture A: Blended FST complexes, prepared with unlabeled T5, were incubated for 10 min in MGM at 37.5° and 10^8 cells/ml. CM was added (50 μg/ml); then, 5 min later, P^{32}-T5 was added. The complexes were incubated for an additional 15 min. Culture B: A second sample of the same blended FST complexes was exposed to CM at 0° for 5 min, diluted in MGM plus CM and incubated for 15 min at 37.5°, superinfected with P^{32}-T5, and incubated for another 15 min. Culture C: Uninfected cells were blended, then treated like culture A.

Experiment 2, Culture A: Blended FST complexes, prepared with unlabeled T5, were incubated for 5 min in MGM at 37.5° and 10^8 cells/ml to allow protein synthesis. They were then chilled, centrifuged, resuspended in buffer at 5×10^9 cells/ml, superinfected with P^{32}-T5, and incubated for 10 min at 37.5°. Culture B: A second sample of the same blended complexes was treated like culture A, except for the 5-min incubation at 37.5° in MGM. Culture C: Uninfected cells were blended, then treated like culture A.

is responsible for inducing the protein synthesis needed for full DNA transfer, the results in Table 1 suggested that DNA transfer from superinfecting phage and stabilization might occur in the presence of CM if (*a*) host cells were infected under FST conditions and blended, (*b*) the retained FST DNA fragments were allowed to induce protein synthesis before CM addition and superinfection, and (*c*) the "transfer" protein could be utilized by phage particles other than the inducing ones. The data in Table 2, experiment 1, confirm this expectation.

The results further suggested that the usual cessation of DNA transfer at the FST stage, when complexes are initiated at 5×10^9 cells/ml in buffer, might be due to lack of protein synthesis under these conditions. If so, transfer of complete DNA molecules and stabilization should occur if blended FST complexes are diluted in MGM to allow protein synthesis, then concentrated to 5×10^9 cells/ml in buffer and superinfected. This prediction is verified in Table 2, experiment 2.

When unblended FST complexes, prepared at 5×10^9 cells/ml, are diluted to about 10^8/ml in nutrient medium, there is a lag of a few minutes before DNA transfer and stabilization increase.[1] Similarly, when complexes are initiated directly at cell concentrations allowing stabilization to proceed, there is an appreciable delay between adsorption and stabilization.[6] If, as now seems probable, these rate phenomena reflect a requirement for intercurrent protein synthesis, stabilization should be accelerated in the presence of presynthesized "transfer" protein. Table 3 confirms this expectation.

Discussion.—The present results show that transfer of the FST section of the T5 DNA molecule proceeds in the absence of protein synthesis. The temperature dependence of this initial step of transfer[7] may mean that a tail-associated enzyme, possibly a lysozyme, has to interact with the host cell before the FST section can enter. Transfer of the rest of the DNA molecule requires the synthesis of one or more proteins that either do not exist in the uninfected cells or are not available to the phage. The mode of action of the "transfer" protein(s) remains speculative but may be analogous to that of the proteins mediating competence in bacterial transformation.[8]

All the results accord with the attractive hypothesis that the FST section itself contains the information for the protein needed to complete DNA transfer. Two

TABLE 3

ACCELERATION OF STABILIZATION IN SUPERINFECTED FST COMPLEXES

Min	Stabilized Complexes, % A. No FST DNA	B. FST DNA
5	3	75
7.5	27	86
10	60	91

Culture A: Blended, uninfected bacteria. Culture B: Blended FST complexes prepared with unlabeled T5. Both cultures were diluted to 2×10^8 cells/ml in MGM at 37.5°. After 5 min incubation, unlabeled phage was added. At different times after phage addition, samples were diluted into chilled buffer and blended. Stabilized complexes are expressed as per cent of their respective unblended controls.

alternatives must, however, be considered: (1) the FST DNA (or some other material concomitantly injected) activates some bacterial genes; (2) crucial bacterial proteins, normally present, are lost during the initiation of infection and must be resynthesized. The second alternative presupposes that phage particles superinfecting blended FST complexes do not cause the same protein loss. Both alternatives seem improbable, since bacterial DNA is rapidly degraded after transfer of FST DNA.[7] However, these possibilities cannot yet be dismissed.

A genetically specific role of the FST section is compatible with evidence indicating that the T5 genetic map is linear,[9] not circular, and that the DNA molecules in a population have the same nucleotide sequence.[10] It would follow that the phage (*a*) injects always the same end of the DNA molecule first, or (*b*) has the same information at both ends of the molecule. If neither of these alternatives is correct, about half of the phage population should be incapable of productive infection but, at least with one strain of T5, this does not appear to be so.[11]

In contrast to T5, the T-even phages have a circular genetic map[12] and their DNA nucleotide sequences appear circularly permuted.[10] If our views are correct, DNA transfer by these phages should *not* be dependent on protein synthesis, and this appears to be the case.[13] In fact, a survey[14] of existing information for various tailed phages suggests a provocative correlation among (*a*) topology of the genetic map (linear versus circular), (*b*) metabolic requirements for DNA transfer to host cells, and (*c*) the anatomical complexity of the phage tail and its probable role in the initiation of infection.

Summary.—T5 transfers about 8 per cent of its DNA molecule to the host cell in the absence of protein synthesis. Transfer of the rest of the molecule occurs only after fresh protein synthesis. It is proposed that the initial DNA segment contains the information for this protein synthesis.

The author is indebted to the members of the Molecular Biology Workshop of this University, and especially to Dr. Frank Lanni, for valuable discussions. The author also thanks Dr. Frank Lanni for editorial help, and Mrs. Shirley Latimer for excellent technical assistance.

* Publication No. 664 from the Division of Basic Health Sciences. This investigation was supported in part by Public Health Service research grant AI 00857, from the National Institute of Allergy and Infectious Diseases.

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A CONJECTURE ON CONFORMATIONS LEADING TO ENERGY TRAPPING IN HELICAL POLYMERS

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Communicated by Raymond E. Zirkle, March 29, 1965

1. *Introduction.*—The observation that solutions of iso- and atactic polystyrene, and atactic polyvinylnaphthalene,[1, 2] all display a structureless emission band shifted $\sim$6000 cm^{-1} to the red from the normal fluorescence without a corresponding band being present in the absorption spectra, and the occurrence of a similar emission from concentrated solutions of many aromatic hydrocarbons,[3] has been interpreted in terms of emission from an "excimer,"[4, 5] a dimeric species which is unstable in the ground state, but is stable in the excited state. Excimer formation leads to energy trapping and is pictured to occur as follows:[4] During the lifetime of a molecular excitation, an excited chromophore and one in the ground state approach sufficiently close for a stable excimer to be formed. The dissociation of the excimer, accompanied by emission of light, is postulated to be responsible for the anomalous fluorescence.

The presence of an excimer band in both atactic and isotactic polystyrene[1] shows that in this example the helical conformation is not especially favorable for excimer formation. Indeed, the observation in the atactic polymer of a slightly greater intensity of the excimer fluorescence relative to the normal fluorescence than in the isotactic polymer, argues for a general nonhelical conformation in the isotactic polymer. Theoretical and experimental investigations of the fluorescence of a series of model compounds with differing chromophore orientation and separation has shown that it should be possible to meet both the conformation and separation requirements for excimer formation between nearest neighbors in polystyrene.[1, 4]

It is possible that energy trapping plays an important role in some biological reactions, and it is therefore worth while to examine the properties of poly-α-amino acids in which the transition from a rigid helical structure to a random coil can be