Mutations in a Nonessential Viral Gene Permit Bacteriophage T4 to Form Plaques on *Escherichia coli valS*^{ts}*relA*

Abstract. *During viral development bacteriophage T4 modifies the valyl-transfer RNA synthetase of its host* Escherichia coli, *but the function of the modification has remained elusive. A strain of* Escherichia coli *has now been identified which is nonpermissive for wild-type bacteriophage T4, but permissive for bacteriophage mutants impaired in the modification reaction. A comparison with other bacteria suggests that nonpermissiveness is due to synthesis of a thermolabile valyl-transfer RNA synthetase and relaxed control of RNA accumulation.*

Bacteriophage T4 normally modifies *Escherichia coli* valyl-transfer RNA (tRNA) synthetase by adding a small, basic peptide, τ, to the host enzyme (*1*). While the phage-modified enzyme is more refractory to inactivation by heat and 4*M* urea, no major alteration in the catalytic parameters of the aminoacylation reaction can be detected (*2*). During infection at 32°C of *E. coli* with a thermolabile valyl-tRNA synthetase, specified by the *valS*^{ts} allele, the τ peptide stabilizes the enzyme and allows virus production at temperatures which are nonpermissive for growth of the uninfected bacteria (*3*). The phage gene *vs* that is responsible for this modification is expressed immediately after infection and is presumed to be the structural gene for the τ peptide (*4*). Bacteriophage T4 *vs* gene mutants, deficient in this modification, grow well on typical laboratory strains of *E. coli* and fail to show even subtle perturbations in the orderly expression of biochemical events that result in viral progeny (*5*).

Table 1. Strains of *E. coli* and bacteriophage T4.

Strain	Source	Description
T4Bc$^+$	This laboratory	Wild-type; normal modification of VRS*
T4*vs1*	This laboratory	Missense mutant; unusual modified VRS
T4*vs2*	This laboratory	Amber mutation; no modified VRS
T4*vs2RF01*	This laboratory	Spontaneous revertant of T4*vs2*; modified VRS
T4Do	D. Hall	Shock resistant derivative of T4D; modifies VRS
T4*tk2*	D. Hall	Deletion; *nrdC* to *tk*; excludes *vs*; modifies VRS
T4*tk3*	D. Hall	Deletion; *rI* to *reg B*; includes *vs*; no modified VRS
T4*tk25*	D. Hall	Deletion; similar to *tk3*; includes *vs*; no modified VRS
T4*farP13*	D. Hall	Deletion; *nrdC* to *denV*; includes *vs*; no modified VRS
E. coli NP 4	This laboratory	Wild-type *E. coli* B; indicator strain
E. coli GM 23	This laboratory	F$^-$ *his metB relA*; by P1 transduction from CP 790302
E. coli KL 16	M. Comer	Hfr *thi-1 rel-1* λ^-
E. coli K 10	M. Comer	Hfr *rel-1 ton A22* T2^R
E. coli JP 1116	M. Comer	HfrH *pheT354 galE-PL-5 rel-1*
E. coli NP 37	M. Comer	Hfr *pheS5 rel-1 tonA22* T2^R
E. coli CP 790302	G. Björk	F$^-$ *pyrB his metB relA valS*^{ts} *rpsL ampA* (*valS*^{ts} by P1 from NP 910212)
E. coli CP 78	G. Björk	F$^-$ *thr leu argH his*
E. coli CP 79	G. Björk	F$^-$ *thr leu argH his relA*
E. coli NP 29	This laboratory	*valS*^{ts}
E. coli NP 910212	This laboratory	F$^-$ *recA strA valS*^{ts} *pyrB trpA*

*Valyl-tRNA synthetase

The *vs* gene is, thus, typical of a large number of T4 genes that are termed nonessential, but constitute approximately 60 percent of the viral genome (*6*). Research on the function of "nonessential" genes in general, and the *vs* gene in particular, has been hampered by an inability to fabricate systems where the biological consequences are obvious. I report here the fortuitous discovery of a second *vs* gene-dependent biological phenomenon: bacteriophage T4 are unable to produce plaques at 32°C on a strain of *E. coli* that simultaneously harbors the *relA* and *valS*^{ts} alleles, unless the bacteriophage contain mutations within or have lost the *vs* gene.

In order to identify strains of *E. coli* suitable for T4 gene cloning experiments, I attempted to titrate lysates of certain bacteriophage strains in our laboratory collection on a number of *E. coli* strains. Table 1 lists the salient characteristics of the bacteria and phage that were used in these experiments. Table 2 is a résumé of the relative plating efficiencies that were obtained. Four strains of bacteriophage, T4Bc$^+$, T4Do, T4*vs2RF01*, and T4*tk2*, were unable to form visible plaques on *E. coli* CP 790302. Their common property is the ability to modify the host valyl-tRNA synthetase. It is noteworthy that T4*vs2RF01* was isolated as a spontaneously occurring revertant of T4*vs2*, and has reacquired some ability to modify the bacterial valyl-tRNA synthetase. Similarly, while T4*tk2* contains a large deletion, from *nrdC* to *tk*, the deletion does not include the *vs* gene function, and modification proceeds normally with this strain.

In contrast, bacteriophage that successfully plate on CP 790302 are uniformly impaired in the only known *vs* gene function, that is, the modification of valyl-tRNA synthetase. Bacteriophage T4*vs1* and T4*vs2* contain, respectively, missense and amber mutations within

Table 2. Relative efficiency of plating bacteriophage T4 on *E. coli*. The data are expressed as the ratios of the number of plaques formed on the indicated host to the number formed on *E. coli* B (NP 4) by the soft agar overlay technique. Absolute values of the lysate titers ranged from 1×10^{11} to 3×10^{11} plaque-forming units per milliliter on the permissive host *E. coli* B (NP 4). Plaques were allowed to develop overnight at 32°C but could be seen after 8 hours of incubation.

Phage	Bacteria									
	GM23	KL16	K10	JP1116	NP37	CP790302	CP78	CP79	NP29	NP910212
T4Bc$^+$	1.20	1.13	1.18	0.60	0.96	$< 4 \times 10^{-4}$	0.75	0.82	0.95	1.16
T4*vs1*	0.81	0.88	0.83	0.53	0.67	0.50	0.89	0.87	0.92	0.75
T4*vs2*	0.90	0.95	1.00	0.41	0.64	0.52	0.75	1.00	0.68	0.79
T4*vs2RF01*	0.59	0.58	0.67	0.43	0.47	$< 6 \times 10^{-4}$	0.73	1.00	0.71	0.81
T4Do	0.51	0.72	0.68	0.40	0.40	$< 4 \times 10^{-4}$			0.39	0.54
T4*tk2*	0.37	0.89	0.81	0.42	0.61	$< 3 \times 10^{-4}$			0.54	0.68
T4*tk3*	0.48	0.69	0.60	0.37	0.38	0.32			0.48	0.73
T4*tk25*	0.53	1.08	0.99	0.31	0.70	0.47			0.81	0.28
T4*farP13*	0.57	0.95	0.97	0.47	1.00	1.03			0.72	0.57

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the *vs* gene; while T4*tk3*, T4*tk25*, and T4*farP13* contain extensive deletions which include the *vs* gene (*7*).

A comparison of the genotypes of the *E. coli* strains indicates that the simultaneous presence of the *relA* and *valS*^{ts} alleles is crucial for the differential plating phenomenon. Introduction of the normal *valS* allele into strain CP 790302 via P1*vir* transduction (GM 23) restores its ability to plate normal phage. An isogenic (except for *relA*) pair of strains, CP 78 and CP 79, also contain the normal *valS* allele and do not exhibit differential plating of normal versus *vs* phage. This is also true for strains KL 16, K 10, JP 1116, and NP 37, which are *rel-1* (isogenic with *relA*) but harbor the wild-type *valS* allele. In addition, since JP 1116 and NP 37 produce thermolabile phenylalanyl-tRNA synthetases, temperature sensitivity of a different aminoacyl-tRNA synthetase is not sufficient for the phenomenon. Finally, while NP 29 and NP 910212 contain *valS*^{ts} alleles they plate all phage efficiently, presumably because they are *relA*$^{+}$.

Whether the inability to plate bacteriophage T4 on strain CP 790302 is dependent on the particular allelic state of the *valS* and *relA* genes remains to be determined. Strains NP 29 and NP 910212 most probably contain different allelic states of the *valS*^{ts} gene as they were isolated independently (*8*). On the other hand, the *valS*^{ts} allele in CP 790302 comes from NP 910212 by P1*vir* transduction (*9*). In contrast, the *relA* alleles in the strains in Table 1 are identical, having been derived from CP 79.

These data thus demonstrate that the simultaneous presence of certain mutations within the *relA* and *valS* genes render *E. coli* nonpermissive for T4 bacteriophage that contain a wild-type *vs* gene. The implication exists, therefore, that the *relA* gene product or subsequent metabolites, for example, ppGpp, may play a role during the development of bacteriophage T4 in *E. coli*. Since the *relA* locus influences a large number of biosynthetic and catabolic systems in bacteria (*10*), and has also been shown to affect the accumulation of RNA in T4-infected *E. coli* (*11*), an explanation of the mechanism of the phenomenon is not possible at this time.

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Myocardial Infarct Imaging of Antibodies to Canine Cardiac Myosin with Indium-111-Diethylenetriamine Pentaacetic Acid

Abstract. *Antibodies, by virtue of marked selectivity and affinity, may lend themselves to identification of structures of unique antigenic specificity in vivo. In experimental myocardial infarction in dogs, F(ab')$_2$ fragments of antibodies to cardiac myosin that had been labeled with iodine-131 were shown to localize within the lesion. Because the energy characteristics of iodine isotopes are not ideal for imaging with a gamma camera, a new method for labeling antibody fragments with divalent or polyvalent radionuclides was developed. A bifunctional chelating agent, diethylenetriamine pentaacetic acid was covalently coupled, by an amide bond, to Fab fragments of antibodies to canine cardiac myosin. A stable chelate was then formed with indium-111, a nuclide that has appropriate half-life and energy characteristics for gamma imaging. Antibodies treated in this way retain their antigen-binding activity and are useful in locating myocardial infarcts in vivo.*

Irreversible ischemic tissue injury results in a loss of integrity of the cell's plasma membrane, as evidenced by leakage of intracellular enzymes from regions of myocardial infarction (*1*). This increase in membrane permeability should also permit the entry of extracellular molecules into the damaged cell. If the extracellular molecule is an antibody specific for an intracellular component, it should concentrate within the injured tissue. We have previously demonstrated that, following experimental myocardial infarction in dogs, ^{125}I-labeled F(ab')$_2$ fragments of antibodies specific for cardiac myosin localize and concentrate within the lesion (*2*). Neither ^{131}I nor ^{125}I are ideal gamma-imaging

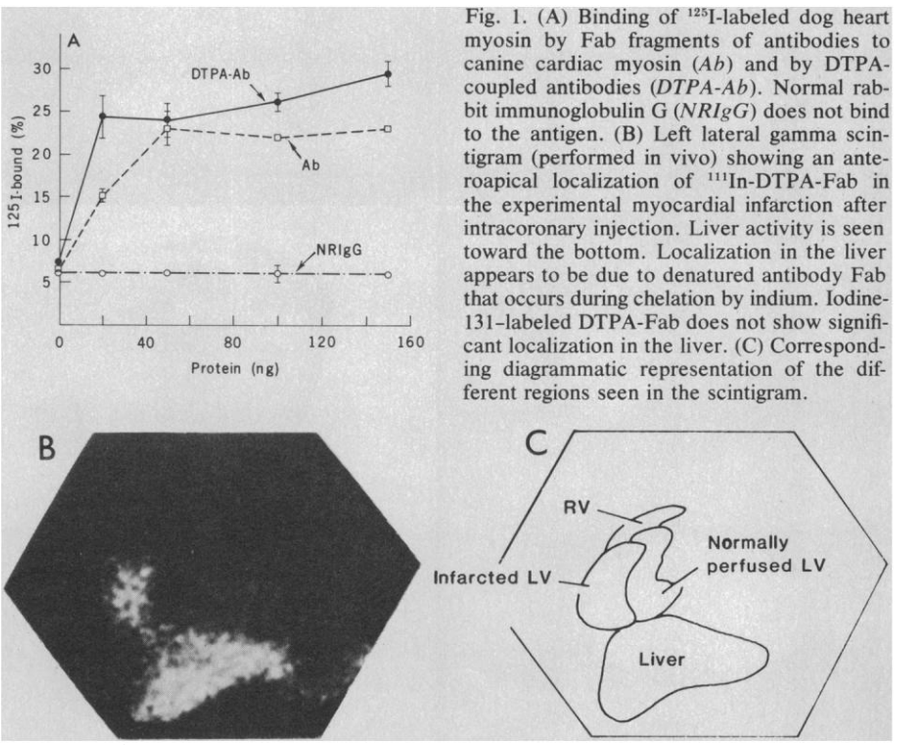

Fig. 1. (A) Binding of ^{125}I-labeled dog heart myosin by Fab fragments of antibodies to canine cardiac myosin (*Ab*) and by DTPA-coupled antibodies (*DTPA-Ab*). Normal rabbit immunoglobulin G (*NRIgG*) does not bind to the antigen. (B) Left lateral gamma scintigram (performed in vivo) showing an anteroapical localization of ^{111}In-DTPA-Fab in the experimental myocardial infarction after intracoronary injection. Liver activity is seen toward the bottom. Localization in the liver appears to be due to denatured antibody Fab that occurs during chelation by indium. Iodine-131-labeled DTPA-Fab does not show significant localization in the liver. (C) Corresponding diagrammatic representation of the different regions seen in the scintigram.

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