JOURNAL OF BACTERIOLOGY, May 1989, p. 2265–2270
0021-9193/89/052265-06$02.00/0

Vol. 171, No. 5

MINIREVIEW

Bacteriophage T4 Genetic Homologies with Bacteria and Eucaryotes

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INTRODUCTION

Although bacteriophage T4 has been one of the most important organisms for studying fundamental problems in molecular biology, there has been little serious consideration of its phylogenetic relationship to the rest of the biological world. Because phage T4 is an obligate parasite of *Escherichia coli*, it might be thought that it is more closely related to bacteria than to eucaryotes. However, the discovery in phage T4 of self-splicing introns (12, 20, 51), which previously had been known to occur only in eucaryotes, suggested a relationship between phage T4 and eucaryotes. The purpose of this article is to review the available evidence for genetic homology between phage T4 and bacteria and eucaryotes and to clarify the phylogenetic relationship of phage T4 to these organisms. Only results that are published or in press are included in this article.

CRITERIA USED TO INFER COMMON ANCESTRY

Although functional and structural comparisons were used in addition to amino acid sequence comparisons to judge common ancestry, amino acid sequence homology was the criterion most frequently employed. The amino acid sequences were all derived from DNA sequence data. In most cases the authors making the comparisons judged that the amino acid sequence homologies were significant and/or implied common ancestry. Doolittle (14) has discussed the criteria for judging whether similar amino acid sequences imply common ancestry. He was especially concerned with those distant relationships in which the sequences being compared fall between 15 and 25% identity, when it is difficult to decide between chance similarity and genuine common ancestry. In this range, the sequence comparison is often insufficient by itself to justify a claim of common ancestry, and it must be supported by ancillary biochemical evidence for functional or structural similarity. In the comparisons summarized below, except as noted, we judged common ancestry to be a reasonable explanation for the similarities observed. This judgement was necessarily inexact since the kinds of evidence used by different authors to infer genetic homology varied widely, with some relying almost entirely on amino sequence comparisons and others emphasizing structural or functional similarity.

Homology due to convergent evolution is distinguishable from ancestry due to descent from a common ancestor. According to Dobzhanzky et al. (13, p. 326), convergent evolution occurs when "two lineages evolve towards morphological similarity under selection to adapt to similar environmental conditions." As a general rule, similarities due to convergent evolution lack the detailed correspondence of parts observed in cases of homology. The examples of homologies listed in Table 1 have sufficiently detailed correspondences to indicate common descent. In particular, it is difficult to explain extended sequence homologies in terms of convergent evolution.

PHAGE T4 GENES HAVING HOMOLOGIES WITH BOTH BACTERIAL AND EUCARYOTIC GENES

Gene 52. Gene 52 encodes one of the three subunits of an ATP-dependent type II topoisomerase (32, 53). Topoisomerase II is postulated to be necessary for initiation of phage T4 chromosome replication (28, 35, 39). Mutants defective in each of the phage topoisomerase genes (genes 39, 52, and 60) can be partially compensated for by the *E. coli* gyrase, suggesting functional similarity (34). The phage T4 gene 52 product (gp52) is only half as large as the *Bacillus subtilis* GyrA subunit (441 versus 821 amino acids) and aligns with only the amino-terminal half of the *B. subtilis* protein. Within this alignment, 32% of amino acids are identical. The homology alignments of phage T4 gp52 with yeast topoisomerase II and *B. subtilis* GyrA protein (Table 1) suggest that a consensus sequence surrounds the reactive tyrosine which is used to form a transient protein-DNA intermediate (24). The phage T4 type II topoisomerase requires ATP for the relaxation of supercoiling but does not catalyze the ATP-dependent introduction of negative supercoiling as do the *B. subtilis* and *E. coli* gyrases. In this regard, the phage T4 type II topoisomerase is functionally more like the yeast than the bacterial type II topoisomerase (24).

Gene 39. gp39, with 520 amino acids, is the largest of the three phage T4 type II topoisomerase subunits. It shares homology with *B. subtilis* GyrB protein and yeast topoisomerase II (Table 1).

Thymidylate synthase gene. The thymidylate synthase gene (*td*) was sequenced by Chu et al. (12). From published sequences of thymidylate synthases (TS) of five eucaryotes and two bacteria, we calculated the percentages of identical matches to the phage T4 TS (Table 1), using the alignments suggested by Taylor et al. (54). In addition, the phage T4 TS had homologies with the TS of herpesvirus saimiri (41%) and *B. subtilis* phage thyP3 (32%). Forty of the 286 amino acids of the phage T4 TS were identical in all of the species examined. The average overall homology to eucaryotes (human, mouse, *Saccharomyces cerevisiae*, *Leishmania major*, and *Leishmania tropica*) was 42%, whereas that to bacteria (*E. coli* and *Lactobacillus casei*) was 43%. By comparison, the TS from eucaryotes (*S. cerevisiae* and human) share 58% identical matches (54), and those from bacteria (*E. coli* and *L. casei*) share 62% identical matches (4). It is interesting that the phage T4 dCMP-hydroxymethylase encoded by gene 42 has significant homology with the TS of phage T4 as well as with the TS of the bacteria *E. coli* and *L. casei* (30).

Dihydrofolate reductase gene. The dihydrofolate reductase gene (*frd*) encodes a 193-residue polypeptide (45). Genes

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TABLE 1. Genetic elements of phage T4 that are homologous to genetic elements of bacteria and eucaryotes

Homologous group(s)	Gene or sequence element in phage T4	General function	Specific function	Eucaryotes		Bacteria		Reference(s)
				Homologous sequence(s)	Evidence for homology	Homologous sequence(s)	Evidence for homology	
Bacteria and eucaryotes	Gene 52	Replication	Topoisomerase subunit	Topoisomerase II (carboxy half) of *S. cerevisiae*	29% identity in aa sequence[a]	GyrA (amino half) of *B. subtilis*	32% identity in aa sequence	24
	Gene 39	Replication	Topoisomerase subunit	Topoisomerase II (N-terminal half) of *S. cerevisiae*	aa sequence comparison noted but not presented	GyrB of *B. subtilis*	35% identity in aa sequence	24, 25
	Gene *td*	Nucleotide metabolism	TS	TS of human, mouse, *S. cerevisiae*, *L. major*, and *L. tropica*	45, 44, 41, 40, and 40% identity, respectively, in aa sequence	TS of *E. coli* and *L. casei*	44 and 42% identity, respectively, in aa sequence	54
	Gene *frd*	Nucleotide metabolism	DHFR	DHFR of human and *L. major*	14 and 16% identity, respectively, in aa sequence	DHFR of *E. coli* and *L. casei*	21 and 18% identity, respectively, in aa sequence	7
Eucaryotes only	Introns of *td*, *nrdB*, and *sunY*	Unknown, possibly regulatory	Self-splicing	Group I introns	Similarity in consensus elements, putative secondary structure, and self-splicing pathway			12, 50
	Gene 43	Replication	DNA polymerase	DNA polymerase of human and *S. cerevisiae*	27 and 21% identical aa, respectively, in conserved regions			52, 63
	Genes 46 and 47	Recombination and repair	Exonuclease	RAD52 of *S. cerevisiae*	Complementation and 26% identical aa in conserved region			10
	Gene 30	Replication and repair	DNA ligase	Ligase of *S. cerevisiae* and *S. pombe*	Within ATP-binding domain, 36 and 21% identical aa, respectively			2, 3
	Gene *e*	Bacterial cell lysis	Lysozyme	Lysozyme of hen and goose	Corresponding three-dimensional structures			60, 61
Bacteria only	Gene *uvsX*	Recombination and repair	Promotes strand exchange			RecA of *E. coli*	Complementation, functional similarity, and 26% identity in aa sequence	17, 38
	Gene 32	Replication and repair	Single-stranded-DNA-binding protein			RecA of *E. coli*	Similar disposition of functional groups	44
	Gene *denV*	Repair	Glycosylase and apurinic endonuclease			Endonuclease of *M. luteus*	Antigenic and functional similarity	D. B. Yarosh and J. Ceccoli, Photochem. Photobiol., in press
	Gene 69	Replication	Unknown			DnaA of *E. coli*	30% identical matches in conserved region	40
	Gene *dam*	Replication	Methyltransferase			*Eco*RV and Dam of *E. coli*, *Dpn*II of *S. pneumoniae*	37, 34 and 35% identical aa, respectively, within conserved regions	31

[a] aa, Amino acid.

encoding dihydrofolate reductases (DHFR) from several other species have been sequenced, including that of *L. major*, which synthesizes a bifunctional enzyme having both DHFR and TS activities. The carboxy-terminal portion of the phage T4 DHFR extends beyond that of the other DHFR sequenced, so that only 165 of the 193 amino acids of the phage T4 DHFR align with the amino acid sequences from the other organisms. Within this region, we determined the phage T4 DHFR percentages of identical matches (Table 1). These percentages were low, but at 12 positions all five species had the same conserved amino acids, indicating that the similarities were not due to chance. These comparisons suggest that the phage T4 DHFR has weak and similar homology to the DHFRs of both bacteria and eucaryotes. The 3′ end of the phage T4 *frd* gene overlaps the *td* gene by four nucleotides; the sequence ATGA includes an opal terminator for the *frd* gene and an initiating triplet for the *td* gene (45). This suggested that the phage T4 *frd* and *td* genes may be evolutionary precursors to the gene which encodes the *L. major* bifunctional DHFR-TS protein (45). However, Beverly et al. (7) pointed out that this hypothesis is not supported by the evidence. The *L. major* DHFR-TS gene is more similar to the unlinked monofunctional DHFR and TS genes of eucaryotes than to the adjacent T4 genes.

PHAGE T4 INTRONS AND GENES HAVING HOMOLOGIES WITH EUCARYOTIC INTRONS AND GENES

Introns. Self-splicing (group I) introns are sequences within mRNA capable of catalyzing both their own excision and the ligation of the resulting free ends of the transcript. Group I introns were first demonstrated in the nuclear large rRNA of *Tetrahymena thermophilia* (9). Subsequently, they were found in fungal mitochondrial rRNA (18, 58) and chloroplast rRNA (48). Group I introns appear to be widespread among eucaryotes (8). A group I intron was discovered in the thymidylate synthase gene of phage T4 by Chu et al. (12). Group I introns have since been found in the phage T4 *nrdB* gene (encoding the phage T4 ribonucleotide reductase small subunit) (20, 51) and in the *sunY* gene (50, 56). Since protein catalysts are unnecessary in the splicing of group I introns in vitro, it has been postulated that the precursor RNA assumes a configuration that facilitates self-splicing. By using site-directed mutation it has been shown that several sequence elements in the *Tetrahymena* rRNA intron are involved in base-paired stem structures required for self-splicing (62). The four consensus sequence elements found in all group I introns have been identified in the *td*, *nrdB*, and *sunY* introns of phage T4 as well (50). Furthermore, the nucleotide sequences of the three phage T4 introns can be represented by a similar secondary structure that conforms with a consensus core secondary structure proposed for group I introns of eucaroytes (37, 50, 59). In addition to these structural similarities, the molecular pathway of intron self-splicing is similar for the phage T4 *td* intron and the *Tetrahymena* rRNA intron (50). Phages T2 and T6 are closely related to phage T4, but both T2 and T6 lack the *nrdB* intron (43), and T6 lacks both the *nrdB* and *sunY* introns (11). This suggests that these introns have been gained or lost, or both, in recent evolutionary time (43).

Gene 43. The DNA polymerase encoded by gene 43 has been sequenced by Spicer et al. (52). The gene 43 polymerase was compared with eight other polymerases (63), including those of six viruses (virus comparisons are not listed in Table 1). Six distinct regions designated I to VI were found to be highly conserved in most polymerases. Five specific regions of the phage T4 gene 43 polymerase (designated I, II, III, IV, and VI), with 220 amino acids, showed amino acid sequence homologies to corresponding regions of the other polymerases. Homologies with human polymerase alpha and yeast polymerase I are shown in Table 1. There were also homologies with polymerases of *B. subtilis* phage φ29 (13%), vaccinia virus (27%), cytomegalovirus (25%), Epstein-Barr virus (25%), herpes simplex virus (24%), and adenovirus type 2 (16%). In a different study (27) only regions I, II, and III, with 74 amino acids, were compared. The percentages of identical amino acids were as follows: herpes simplex virus, 32%; vaccinia virus, 30%; phage φ29, 28%; Epstein-Barr virus, 28%; adenovirus type 2, 24%; and phage PRD1, 24%. The phage T4 DNA polymerase has very little similarity to *E. coli* DNA polymerases I and III and no detectable similarity to phage T7 DNA polymerase (52). The conserved regions in the group of DNA polymerases with homology to the phage T4 polymerase appear to be the active sites for interaction both with deoxyribonucleotides and with DNA (63). Bernad et al. (5) found that the phage T4 DNA polymerase was highly sensitive to the drug aphidicolin and the nucleotide analogs butylanilino dATP and butylphenyl dGTP, which are known to be specific inhibitors of eucaryotic alphalike polymerases. The Klenow fragment of *E. coli* polymerase I was found to be insensitive to these inhibitors.

Genes 46 and 47. The *RAD52* gene of *S. cerevisiae* and the 46-47 gene pair of phage T4 are essential for most recombination events in their respective organisms. When the yeast *RAD52* gene is allowed to express in *E. coli*, it complements phage T4 mutants defective in gene 46 or gene 47 with respect to progeny production, ability to carry out recombination of genetic markers, and recombinational repair (10). The sequences of genes 46 and 47 (21) were compared with the sequence of the *RAD52* gene (1) (Table 1). The gp46 sequence included a patch of 40 contiguous amino acids which had 11 identical matches and an additional 21 conservative substitutions in common with a similar 40-amino-acid patch in RAD52, giving 80% identical-plus-conservative matches (see reference 16 for a discussion of conservative matches).

Gene 30. Gene 30 encodes an ATP-dependent DNA ligase which is important during replication (41, 42, 46), recombination (6), and DNA repair (6) processes. It forms a ligase-AMP complex which catalyzes phosphodiester bond formation between adjacent 3′-hydroxyl and 5′-phosphate groups in double-stranded DNA (46). Its amino acid sequence has been compared with the amino acid sequences of the ligases of *S. cerevisiae* and phage T7 (2). In a 42-amino-acid segment, probably an ATP-binding domain, there were homologies with *S. cerevisiae* (Table 1) and there was 24% identity with the similar small segment of phage T7 ligase. We also compared the phage T4 ligase amino acid sequence with that of *Schizosaccharomyces pombe* (3) and found similar homologies (Table 1).

Gene *e*. Gene *e* encodes a lysozyme which degrades bacterial cell walls. Four families of lysozymes have been identified: hen egg white (HEWL group), goose egg white (GEWL group), phage T4 (T4L group), and bacterial (BL group). Within, but not between, each family the amino acid sequences are clearly related (49). Within the T4L group, T4 lysozyme has 23% identical matches with *Salmonella typhimurium* phage P22 lysozyme and 22% identical matches with *B. subtilis* phage φ29 lysozyme (19). The phage φ29 lysozyme is able to complement a phage T4 *e* mutant (49). The three-dimensional structures of GEWL, HEWL, and T4L, determined at high resolution by X-ray crystallography, have very high correspondences, leading Weaver et al. (60)

to suggest that they diverged from a common evolutionary precursor (Table 1). It was also suggested that the structure of the phage P22 lysozyme provides an evolutionary link between phage T4 lysozyme and GEWL (61).

PHAGE T4 GENES HAVING HOMOLOGY WITH BACTERIAL GENES

Gene *uvsX*. *uvsX* is important in genetic recombination, DNA repair, and replication (6). The purified *uvsX* gene product catalyzes in vitro reactions related to general recombination, similar to the RecA protein of *E. coli* (64). Homology between UvsX and the RecA protein of *E. coli* was reported (Table 1). The conserved sequence proposed for the ATP-binding site of ATPases is found at similar distances from the amino termini of the two proteins (17). Minagawa et al. (38) showed that the *uvsX* gene, borne on a plasmid, functionally substituted for *recA*, rendering *recA* mutant cells more resistant to UV light as well as raising the recombination frequency of phage in *recA* mutant cells. The function of UvsX was significantly enhanced by the presence of UvsY. These results imply that UvsX, particularly in combination with UvsY, can partially substitute for the RecA protein with respect to its role in DNA repair and recombination.

Gene 32. Prashad and Chu (44) have compared six single-stranded-DNA-binding proteins, including phage T4 gp32, *E. coli* RecA, Ssb, and Ssf, phage M13 gp5, and phage Ike gp PIKE. Using gp5 as a template, Prashad and Chu searched for domains in other single-stranded-DNA-binding proteins that yielded the best alignments of aromatic amino acids (Phe, Tyr, and Trp) and basic amino acids (Lys, Arg, and His). Such residues are thought to play a role in interactions with DNA. The identified domains showed alignment of five aromatic and four charged residues in these proteins. Prashad and Chu concluded that these proteins exhibit similar dispositions of functional groups probably involved in single-stranded DNA binding. The RecA protein region between residues 225 and 312 falls into the pattern described by gp5, PIKE, and gp32 in terms of stacking and electrostatic interaction (Table 1). Thus, the *E. coli* RecA protein may share homology with both phage T4 gp32 and phage T4 UvsX (see previous section).

Gene *denV*. *denV* encodes endonuclease V. DenV participates in the repair of pyrimidine dimers in UV-irradiated DNA by cleavage of the 5′-glycosyl bond of the dimer and subsequent endonucleolytic incision of the apyrimidimic site. The DNA repair endonuclease of the bacterium *Micrococcus luteus* produces breaks in DNA in the same way and is similar in size, stability, salt and pH dependence, metal independence, and substrate specificity. Antigenic determinants of these two enzymes are also shared (Table 1). The protein sequence comparison must await cloning and sequencing of the *M. luteus* gene.

Gene 69. gp69 is involved in phage DNA replication and has homology with a segment of the *E. coli* DnaA protein which is required for initiation of *E. coli* DNA replication (Table 1).

Gene *dam*. *dam* encodes a DNA methyltransferase that methylates the adenine residue in the target sequence GATC. This enzyme has homologies within a 119-amino-acid conserved region (Table 1) with the GATATC-modification methyltransferase *Eco*RV of *E. coli*, as well as with the GATC-recognizing methyltransferases from *E. coli* (Dam) and *Streptococcus pneumoniae* (*Dpn*II) (23, 31).

OTHER HOMOLOGIES

The homologies described in this section are mentioned separately because they do not appear to clarify the ancestral relationships of phage T4.

β-Glucosyltransferase. The phage T4 β-glucosyltransferase was reported to have some sequence similarity with proteins of the leukocyte interferon family but not enough to indicate a structural relationship (55).

Gene 38. gp38 occurs at the tip of the long tail fibers of phage T2 (related to phage T4). It promotes recognition of specific proteins on the outer membrane of the *E. coli* host. Riede et al. (47) have found amino acid sequences in gp38 that are similar to those in OmpA and other outer membrane proteins. They proposed a model for complementary intraction of gp38 and OmpA, and they suggested that the sequence similarities between the phage and bacterial proteins may have arisen by convergent evolution or that the phage may have picked up parts of the relevant *E. coli* genes as a means of improving infectivity.

Gene 55. gp55 of phage T4 endows the *E. coli* RNA core polymerase with the ability to recognize T4 late promoters. gp55 is analogous in function to the much larger sigma-70 protein of *E. coli*, and it has been shown by Gribskov and Burgess (22) to share a region of weak homology with sigma-70 as well as with five other similar proteins from *E. coli*, *B. subtilis*, and phage SPO1. However, the degree of homology between gp55 and the other proteins was so low (e.g., sigma-70 and gp55 shared only 16% identical amino acids in this region) that we have not included it in Table 1.

CUUCGG-containing hairpins. The mRNA of phage T4 contains abundant intercistronic hairpins with a characteristic CUUCGG sequence in the loop. The UUCG sequence dramatically stabilizes the hairpins (57). A bacterium (*E. coli*) and eucaryotes (*Caenorhabditis elegans* and human) also have CUUCGG-containing hairpins. The similarity over such a short region may be due to convergent evolution rather than common descent.

CONCLUSIONS

Table 1 shows that the numbers of reported genetic homologies between phage T4 and bacteria and between phage T4 and eucaryotes are similar. (No comparisons have been made with archaebacteria [15].) The only reported nonhomology of a T4 enzyme and a corresponding bacterial enzyme that we are aware of is between the phage T4 DNA polymerase and the DNA polymerases I and III of *E. coli*. The four phage T4 genes which share homologies with both eucaryotes and bacteria (genes 52, 39, *td*, and *frd*) show about equal similarities to each class.

The simplest interpretation of the evidence reviewed here is that phage T4 shares ancestry with both bacteria and eucaryotes and has about equal similarity to each. Phage T4 may have diverged from a common ancestor of bacteria and eucaryotes or from an early-evolved member of the bacterial or eucaryotic lineage. This conclusion does not take into account the possible effects of such factors as different rates of evolution of the bacterial and eucaryotic lineages or horizontal transfer of genes between bacteria or eucaryotes and the phage genome. It is likely that bacterial evolution has been more rapid (due to a shorter generation time) than eucaryotic evolution (29). Therefore, if the phage T4 lineage emerged from a common ancestor of both bacteria and eucaryotes, it should today be more distant from bacteria than from eucaryotes. Since phage T4 seems about equally distant from both, perhaps the phage ancestral line diverged

from the bacterial line some time after the bacterial and eucaryotic lines diverged from each other.

Michel and Dujon (36) have noted homologies between the amino acid sequence potentially encoded by the intron in the thymidylate synthase gene of phage T4, the intron in the gene encoding NADH dehydrogenase subunit 1 of *Neurospora crassa*, and the first and third introns of the corresponding gene in *Podospora anserina*. They suggested that possibly the phage T4 *td* intron was exchanged relatively recently between a close ancestor of the *Neurospora-Podospora* group of filamentous fungi and the phage T4 genome. Jacquier and Dujon (26) have described an intron-encoded protein which acts in a gene conversion-like process in *S. cerevisiae*. This protein apparently introduces double-strand breaks in a recipient 21S rRNA gene, thus promoting transfer of the intron (33). As a consequence of transfer occurring in heterozygotes, this mechanism causes rapid spread of the intron into copies of the mitochondrial gene throughout an interbreeding yeast population. Although a similar process might be involved in a possible horizontal transfer mechanism, there is currently no evidence for such a mechanism operating between species. In most of the cases summarized here, the low level of sequence homology between the similar phage T4 and eucaryotic genes implies that horizontal transfer, if it occurred, must have been an early evolutionary event. In such cases it is simpler to assume direct descent from a common ancestor.

Most of the phage genes listed in Table 1 act in the ubiquitous processes of DNA replication, DNA repair, recombination, and nucleotide synthesis. Enzymes involved in such fundamental processes probably evolved very early. Adaptive features of these early enzymes may have been preserved in the phage T4, bacterial, and eucaryotic lineages because they were already well-tested solutions to basic functional problems by the time these lineages diverged. The preservation of such adaptive features probably accounts for most of the homologies retained to the present day. Understanding achieved about the experimentally accessible phage T4 gene products thus is likely to be relevant to the homologous eucaryotic gene products. Studies of phage T4 therefore should continue to give useful insights into the fundamental processes of DNA replication, DNA repair, recombination, nucleotide synthesis, and intron processing in eucaryotes.

ACKNOWLEDGMENTS

We gratefully acknowledge the helpful suggestions of S. T. Abedon, P. Y. Cheah, P. Hyman, R. P. McCreary, and R. Kandell.

This work was supported by Public Health Service Biomedical Research Support grant 2S07 RR05675 from the National Institutes of Health.

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