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Genetics

Yeast gene *RAD52* can substitute for phage T4 gene *46* or *47* in carrying out recombination and DNA repair

(recombinational repair/*Saccharomyces cerevisiae*)

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ABSTRACT **The *RAD52* gene of *Saccharomyces cerevisiae* and genes *46* and *47* of bacteriophage T4 are essential for most recombination and recombinational repair in their respective organisms. The *RAD52* gene was introduced into expression vectors that were used to transform *Escherichia coli*. The expression of *RAD52* was then induced, and the ability of *RAD52* to complement phage mutants defective in gene *46* or *47* was determined with respect to the three criteria of phage growth, recombination, and recombinational repair. *RAD52* gene expression was found to allow growth of gene *46* and *47* mutants under otherwise restrictive conditions, as measured by plaque formation and burst size. Expression of the *RAD52* gene also restored the ability of gene *46* and gene *47* mutants to undergo recombination of *rII* markers. Furthermore, the *RAD52* gene restored the ability of gene *46* and *47* mutants to undergo recombinational repair after UV irradiation. The published DNA sequence of gene *RAD52* was compared with the published sequences of genes *46* and *47*. Although overall sequence similarities were only marginally significant, *RAD52* and gene *46* had substantial sequence similarity over a limited region.**

The *RAD52* gene of the yeast *Saccharomyces cerevisiae* and the *46/47* gene pair of bacteriophage T4 are essential for most recombinational events in their respective organisms. Table 1 summarizes the properties of these genes, indicates their functional similarity, and lists the appropriate references. The *RAD52* gene controls an exonuclease, and a functional *RAD52* gene is required for meiotic and mitotic recombination in yeast. It is also required for recombinational repair and production of viable meiotic spores. Genes *46* and *47* code for an exonuclease, possibly composed of two polypeptide chains. This exonuclease expands DNA nicks into gaps, which allows joint molecules to form (1, 6). It is required for most recombination, recombinational repair, and production of viable progeny phage.

We show here that when the yeast *RAD52* gene is expressed in *Escherichia coli* it complements mutants of phage T4 defective in gene *46* or *47*. Complementation is observed with respect to progeny phage production, the ability to carry out recombination of genetic markers, and recombinational repair. A comparison of the published DNA sequence of the *RAD52* gene with each of those of genes *46* and *47* indicated marginally significant overall sequence similarities but substantial sequence similarity between *RAD52* and gene *46* over a limited region. Thus gene *RAD52* and genes *46* and *47* appear to have a common ancestry on the basis of genetic complementation and partial sequence homology.

MATERIALS AND METHODS

The bacterial strains used were *E. coli* S/6, K594(λ), JM105 [*thi*, *rpsL*, *endA*, *sbcB15*, *hspR4*, Δ(*lac–proAB*), (*F'*, *traD36*, *proAB*, *lacI*^{q}ZΔM15)], and N4830-1 [SI, Δ8, (λΔBAM, ΔH1)]. The *RAD52*-containing plasmid YRp7-A4Sal-[RAD52] was kindly given to us by D. Schild (Lawerence Berkeley Laboratory, Berkeley, CA), and the RAD52-containing plasmid YpSL1-[RAD52] was kindly provided by H. Ogawa (Osaka University, Osaka, Japan). Their properties are described in Schild *et al.* (30) and in Adzuma *et al.* (31), respectively. The DNA fragment containing the *RAD52* gene from the first plasmid was removed by *Sal* I restriction enzyme digestion and was subcloned into expression vector pUC18. The *RAD52* gene from the second plasmid was subcloned, also after *Sal* I digestion, into expression vector $pP_{LC}2819$. The expression of *RAD52* in pUC18 is under control of the isopropyl β-D-thiogalactoside (IPTG)-inducible *lacZ* promoter (32). In $pP_{LC}2819$ the *RAD52* gene is under the control of a thermoinducible λ P_L promoter (33). Recombinant plasmids pUC-[RAD52] and pP_{LC}-[RAD52] were used to transform *E. coli* strains JM105 and N4830-1, respectively. *E. coli* JM105, a restrictionless host strain, was developed to allow replication of high-copy number pUC plasmid cloning vectors (34). Strain N4830-1, a λ lysogen, carries the *cIts857* and *N* genes to give a complete thermoinducible protein expression system (35). The procedures used for cloning and turning on expression of the cloned *RAD52* gene were as described in Maniatis *et al.* (36). For induction of the *lacZ* inducible promoter, cells were grown to a density of 2×10^7 per ml, IPTG was added, and growth was continued until the cells reached a density of 2×10^8 per ml.

To detect expression of the *RAD52* gene product, 1 ml of the induced and 1 ml of the uninduced cultures were pelleted in an Eppendorf centrifuge tube and were washed twice with an equal volume of M9 salt solution (15). The pellet was next resuspended in 200 μl of $NaDodSO_4$/PAGE loading buffer (37). The suspension was boiled for 5 min and was cooled, and then 20 μl of each separate sample was loaded in a lane of an electrophoresis apparatus. The $NaDodSO_4$/PAGE analysis was carried out by using modifications of the methods of Laemmli (38) and of O'Farrell and Gold (39). The Bio-Rad $NaDodSO_4$/PAGE protean apparatus with a discontinuous buffer system was used with a slab gel composed of a 4.5% stacking gel and a 12.5% separating gel.

The phage strains used in this study were *rIIA* cistron mutants *rED144* and *r71*; gene *46* mutants *tsL109*, *amNG280*, and *amNO24*; gene *47* mutants *tsL86*, *tsA52*, and *amNO11*. The gene assignments of *tsL109(46)*, *tsL86(47)*, and *tsA52(47)* were verified by the inability of each mutant to complement an amber mutant defective in the same gene. The cross procedures employed for marker recombination were the standard ones described by Bernstein (2, 40). For UV irradiation, phage at about 2×10^{10} per ml were distributed in 10-ml samples in Petri dishes (10). A 15-W General Electric G15T8 germicidal lamp was used at a distance of 23 cm to deliver about 7.5 erg (1 erg = 1.0×10^{-7} J) per mm^2 per sec.

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Abbreviations: IPTG, isopropyl β-D-thiogalactoside; MR, multiplicity reactivation.

Table 1. Comparison of phage T4 genes *46* and *47* with yeast gene *RAD52*

Phenotypic properties of genes *46* and *47*	Refs.	Phenotypic properties of *RAD52*	Refs.
Essential for most recombinational events	1–5	Essential for meiotic and mitotic recombination	17–20
Exonuclease: extends single-strand nicks into gaps	6, 7	Controls an endo–exonuclease	21–24
Repair of DNA damage	8–15	Repair of DNA damage, especially double-strand breaks	19, 25–29
Mutants produce inviable phage with partial genomes	16	Mutants produce inviable spores	17, 18, 25, 26

The procedures used in the multiplicity reactivation (MR) experiments were as described by Chen and Bernstein (15). Briefly, the monocomplex surviving fractions plotted in Fig. 1 were measured in the following way. A sample of 0.5 ml of phage (irradiated or unirradiated) at 2×10^5 per ml was mixed with 0.5 ml of bacteria at 2×10^8 per ml to give a multiplicity of infection of 0.001. At each UV dose, the monocomplex surviving fraction was calculated as the titer of infective centers formed by the irradiated phage divided by the titer of infective centers formed by the unirradiated phage. Multicomplex surviving fractions were measured in the same way except that the irradiated or unirradiated phage were added at 2×10^9 per ml to give an expected multiplicity of infection of 10.

RESULTS

Expression of *RAD52* Gene Product from pUC-[RAD52]. To determine if the *RAD52* gene product is expressed from the pUC-[RAD52] recombinant plasmid, crude protein samples were extracted from *E. coli* JM105 cells containing the plasmid. These were prepared as described (see *Materials and Methods*) and were subjected to 12.5% $NaDodSO_4$/PAGE. A 51-kDa protein was present in cells induced with IPTG but not in cells lacking the plasmid or in uninduced cells. Adzuma *et al.* (31) have estimated the molecular mass of the *RAD52* gene product to be about 52 kDa. Thus it appears that the *RAD52* gene is expressed from pUC-[RAD52].

Complementation with Respect to Phage Growth. *E. coli* JM105 containing recombinant plasmid PUC-[RAD52] was tested for its ability to support plaque formation by gene *46* mutant *tsL109* and by gene *47* mutants *tsL86* and *tsA52* (Table 2). At the permissive temperature of 28°C, these mutants formed plaques on JM105(*RAD52*) whether or not the *RAD52* gene was induced. At the restrictive temperature of 42°C, these mutants did not form plaques on either *E. coli* S/6 or uninduced JM105(*RAD52*). Plaques were formed at 42°C, however, when the *RAD52* gene was induced with IPTG. The efficiency of plating of the temperature-sensitive mutants at 42°C in the presence of expressed *RAD52* varied from 41% to 98% of that at 28°C (Table 2). These results indicate that the yeast *RAD52* gene complements mutants defective in either gene *46* or *47*.

A complementation test was also performed by using the *RAD52* gene from H. Ogawa. This gene was inserted into plasmid $pP_{LC}2819$ so that expression of *RAD52* came under the control of the thermoinducible λ P_L promoter. In this system, *RAD52* is inducible only at high temperatures. In this case amber rather than temperature-sensitive mutants were used in the complementation tests. Two gene *46* mutants, *amNG280* and *amNO24*, and one gene *47* mutant, *amNO11*, were plated on *E. coli* N4830-1(*RAD52*) to measure their plaque-forming abilities. All three amber mutants formed plaques on this host at 42°C when *RAD52* was thermoinduced but not at 28°C when it was not induced. Thus the *RAD52* isolate of H. Ogawa, like the one from D. Schild, complemented phage gene *46* and *47* mutants with respect to plaque formation.

Complementation with Respect to *rII* Marker Recombination and Burst Size. Recombination was measured in crosses between the *rII* markers *r71* and *rED144* in a gene *46* mutant (*tsL109*), a gene *47* mutant (*tsL86*), or a wild-type background (Table 3). The crosses were carried out at the semirestrictive temperature of 37°C for the gene *47* mutant and at 40°C for the gene *46* mutant. The host bacteria, JM105, contained either no *RAD52* plasmid, a *RAD52* plasmid that was uninduced, or a *RAD52* plasmid that was induced. The percent recombination between the *rII* markers in a wild-type background when *RAD52* was not present or uninduced varied from 0.96% to 1.22% (mean = 1.07%). When the phage carried the *tsL109* mutation, the percent recombination was much lower; it varied from 0.01% to 0.06% (mean = 0.04%). When *tsL86* was present, the percent recombination varied from 0.02% to 0.12% (mean = 0.06%). These low percentages of recombination in the presence of either the gene *46* or *47* mutation under semirestrictive conditions reflect the key role of the gene *46/47* exonuclease in recombination. When the plasmid containing the *RAD52* gene was present and induced, the percent recombination in the *tsL109* mutant cross increased 35-fold (1.15–1.47%, mean = 1.31%). Similarly, when the *RAD52* gene was expressed in the *tsL86* mutant cross, the

Table 2. Plaque formation by gene *46* and *47* temperature-sensitive mutants after IPTG induction of *RAD52*

Phage T4 mutant	*E. coli* host	IPTG induced	Plaque formation 28°C	Plaque formation 42°C	% recovery, 42°C/28°C
tsL109 (*46*)	S/6	No	+	−	0
	JM105 (*RAD52*)	No	+	−	0
	JM105 (*RAD52*)	Yes	+	+	98
tsL86 (*47*)	S/6	No	+	−	0
	JM105 (*RAD52*)	No	+	−	0
	JM105 (*RAD52*)	Yes	+	+	41
tsA52 (*47*)	S/6	No	+	−	0
	JM105 (*RAD52*)	No	+	−	0
	JM105 (*RAD52*)	Yes	+	+	58

Table 3. Percentage recombination between phage T4 *rII* mutants in the presence or absence of induced gene *RAD52*

Phage cross	% recombination		
	No plasmid	Uninduced *RAD52* plasmid	Induced *RAD52* plasmid
rED144 × *r71*	0.96, 1.01	1.07, 1.22	1.40, 1.49
rED144, tsL109(46) × *r71, tsL109(46)*	0.01, 0.05	0.03, 0.06	1.15, 1.47
rED144, tsL86(47) × *r71, tsL86(47)*	0.02, 0.12	0.02, 0.08	0.50, 0.67

The two values shown are from separate experiments.

percent recombination increased 10-fold (0.50–0.67%, mean = 0.59%). These increases upon *RAD52* gene induction show that the *RAD52* gene can complement gene *46* and *47* mutants with respect to their defective recombination phenotype.

The mean burst sizes measured in these two crosses are shown in Table 4. In the crosses that are wild-type with respect to genes *46* and *47*, the mean burst sizes varied from 68 to 114 (overall mean = 98) phage per cell. In the *tsL109* (gene *46*) mutant crosses, when *RAD52* was absent or uninduced, the mean burst sizes were 12 and 14, respectively. However, the mean burst size increased 10-fold to 126 when *RAD52* was induced. A 5-fold increase was seen in the *tsL86* (gene *47*) crosses when *RAD52* was induced. These results show that *RAD52* complements gene *46* and *47* mutants with respect to burst size. Thus complementation is evident with respect to two measures of phage growth—efficiency of plating (previous section) and burst size.

Complementation with Respect to DNA Repair. MR is a major form of DNA repair in phage T4 (see ref. 41, for review). This type of repair depends on the presence of at least two phage genomes in the same infected cell and the expression of genes required for recombination. These genes include genes *46* and *47* .

The MR obtained with wild-type phage when the *RAD52* gene is uninduced is shown in Fig. 1*A*. The open circles represent fractional survival after UV irradiation of the infective center-forming ability of cells infected by wild-type phage at a multiplicity of 0.001 (so that most infected cells receive only a single phage genome). The filled circles represent survival of the infective center-forming ability of cells infected by the same irradiated phage but at a multiplicity of about 10. The slower rate of inactivation upon multiple infection compared to single infection reflects MR. When the same experiment is carried out with the *tsL109* (gene *46*) mutant, the rate of inactivation in the multiple infections (filled circles) is distinctly greater than when wild-type phage were used, which indicates reduced MR (Fig. 1*B*). The *tsL86* (gene *47*) mutant also has reduced MR compared to wild-type (Fig. 1*C*). The MR levels obtained when the *RAD52* gene is induced are shown in Fig. 1 *D–F*.

Table 4. Burst size of phage T4 *rII* mutants in the presence or absence of induced *RAD52*

Phage cross	Burst size		
	No plasmid	Uninduced *RAD52* plasmid	Induced *RAD52* plasmid
rED144 × *r71*	68	112	114
rED144, tsL109(46) × *r71, tsL109(46)*	12	14	126
rED144, tsL86(47) × *r71, tsL86(47)*	9	19	67

Each value represents the average of two independent measurements.

The levels of MR in infections by wild-type (Fig. 1*D*), *tsL109* (Fig. 1*E*), and *tsL86* (Fig. 1*F*) were raised substantially compared to that observed when the *RAD52* gene was not expressed. This indicates that the *RAD52* gene not only complements the gene *46* and *47* mutants with respect to MR but that it also increases the efficiency of MR in a wild-type infection. Since MR is a form of recombinational repair, these results imply that the *RAD52* gene can substitute for genes *46* and *47* with respect to their role in recombinational repair, as well as enhance recombinational repair of wild-type.

Comparison of DNA Sequences. The published sequences of genes *46* and *47* (42) were compared to the published sequence of the *RAD52* gene (31) by computer analysis [Rapid Biosequence Similarity Analysis with IBM-PC and PC/XT (43)]. Although in both of these comparisons significant homology was found, in neither case was the overall homology impressive. However, when amino acids 14–199 of gene product 46 were aligned with amino acids 37–334 of *RAD52* gene product and gaps in the sequence were allowed in order to maintain optimum sequence similarity, there were 26% identical matches. Within this region, gene *46* and *RAD52* showed substantial sequence similarity over a contiguous 40-amino-acid region in which there were no gaps in either sequence. This might indicate a uniquely conserved functional region. The ":" and the "." symbols shown in the comparison of these 40-amino-acid sequences indicate identical matches and conserved substitutions, respectively (44).

T4 gene product 46

```
160        170        180        190        199
EDLLEVGTLAEMDKLNKALIRELNSQNQVLDVKKDSIIQQ
.:::. . . . : .....  :.....:.....:.....
DDLLDDSLMFSDDFQDDDLINMGNTNSNVLTTEKDPVVAK
      300        310        320        330
```

Yeast gene product RAD52

DISCUSSION

We have shown here that the yeast *RAD52* gene can substitute for phage T4 genes *46* and *47* with respect to their roles in phage growth, marker recombination, and recombinational repair. We have also shown that *RAD52* has partial sequence identity with gene *46*. These findings suggest that genes *RAD52* and *46/47* may have a common ancestry. Recombination and recombinational repair in phage T4 require at least five genes in addition to genes *46* and *47* (genes *32*, *59*, *uvsW*, *uvsX*, and *uvsY*; see ref. 45 for review). The requirement for at least seven gene products suggests that recombination is carried out by a complex protein apparatus (46). A similar complex protein apparatus may exist in yeast, which is composed of the products of genes in the *RAD52* epistatic group (47). Our finding that the *RAD52* gene can efficiently replace phage genes *46* and *47* indicates that the *RAD52* gene product is able to integrate its action with the rest of the phage recombination apparatus. This suggests that the similarity in the yeast and phage recombination mechanisms may extend beyond the exonuclease function.

Chow and Resnick (23) have proposed that the *RAD52* gene product controls an endo–exonuclease, which they refer to as yNucR. They found that antiserum raised against a purified single-stranded DNA-binding DNase from *Neurospora crassa* identified yNucR as a 72-kDa protein present in wild-type yeast that was reduced in the mutant *rad52-1*. Adzuma *et al.* (31) cloned a yeast DNA sequence which complemented *rad52* mutants. The molecular mass of the *RAD52* gene product, indicated by their sequence data, was about 52 kDa (the exact molecular mass was uncertain because several start codons were possible). Their estimate does not match the 72-kDa molecular mass of the protein identified by Chow and Resnick. The discrepancy between

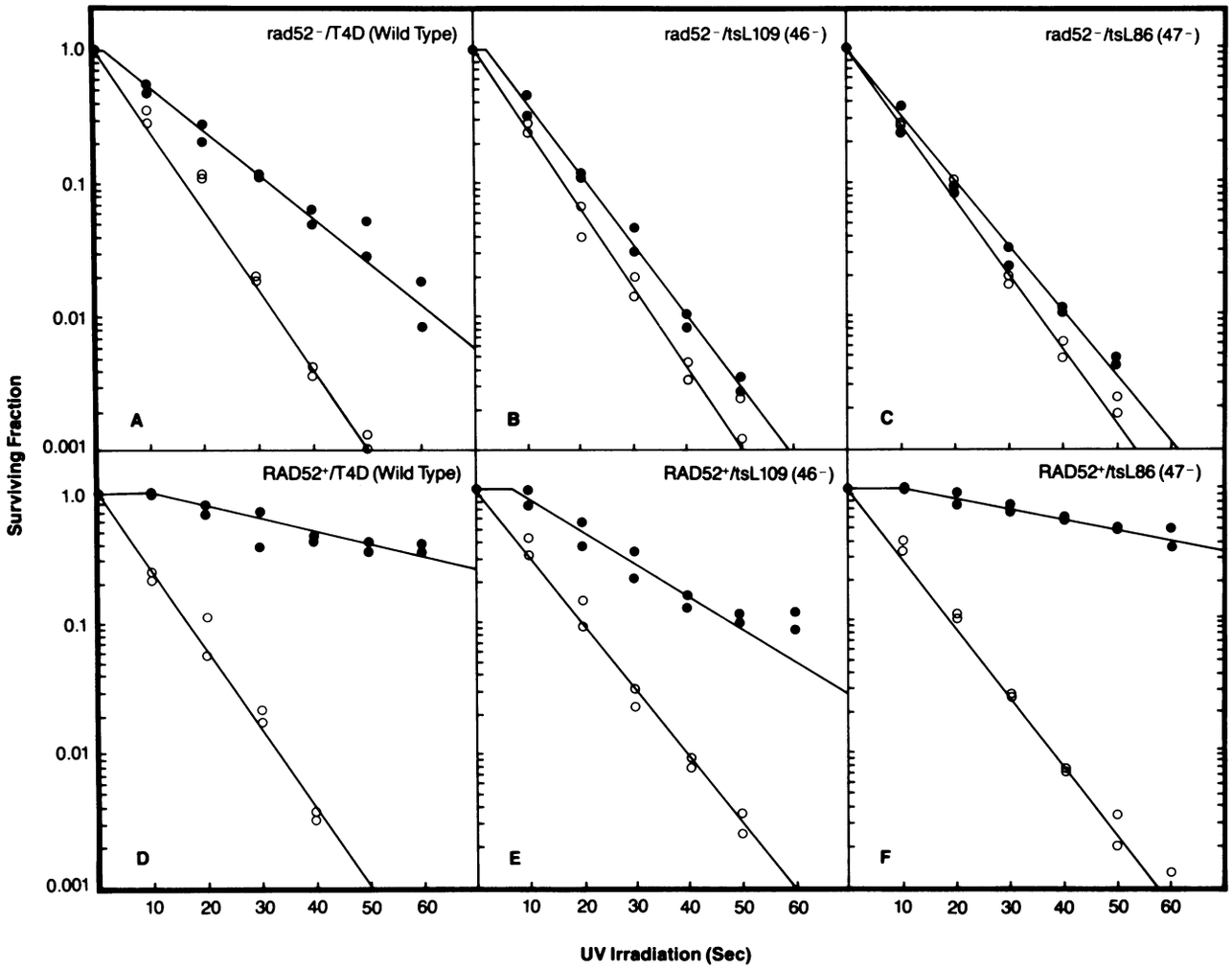

FIG. 1. Comparison of wild-type and mutant monocomplex and multicomplex surviving fractions as a function of UV dose. ○, Monocomplex surviving fractions; ●, multicomplex surviving fractions. (*A–C*) Infections of *E. coli* JM105 containing pUC-[RAD52] that was not induced. (*D–F*) Infections of *E. coli* JM105 containing pUC-[RAD52], where the cells were induced prior to infection. The presence of wild-type alleles or mutant alleles of genes *46* or *47* of the infecting phage is indicated in each panel. Each data point represents the mean value obtained in two independent experiments.

the reported molecular masses may indicate that the DNA sequence isolated by Adzuma *et al.* (31) does not contain the complete *RAD52* sequence or that the *RAD52*-encoded protein and the 72-kDa protein are not the same. Since the DNA fragment isolated by Adzuma *et al.* (31) complements gene *46* and *47* mutants of phage T4, it may possibly encode a gene product that functions as an exonuclease. Recently, Chow and Resnick (24) presented evidence that *RAD52* is not the structural gene for yNucR and suggested that the *RAD52*-encoded protein regulates or activates the yNucR protein. Thus, the *RAD52* gene product may function both to control yNucR and as an exonuclease.

In addition to *RAD52*, two other repair genes have been shown to functionally substitute across the prokaryote–eukaryote boundary. The *denV* gene of phage T4 can complement *RAD1* and *RAD3* mutants of yeast (48) and human Xeroderma D cells (49). The *denV* gene product, endonuclease V, combines a DNA glycosylase activity specific for pyrimidine dimers and an apyrimidinic endonuclease activity in the same molecule. Also, an *E. coli* gene, *ada*, which encodes O^6-methylguanine methyltransferase, has been shown to restore cellular resistance to alkylating agents in repair-deficient human cells (50). Neither of these genes, however, are involved in recombination.

It can be noted in Fig. 1, by comparing *A* to *D*, that multiplicity reactivation of UV-irradiated wild-type phage is more efficient when the *RAD52* gene is induced than when it is not. This suggests that the *RAD52* gene product can promote recombinational repair of UV-induced lethal lesions beyond those handled by the normal phage T4 recombinational repair apparatus. This may be a quantitative effect resulting from overproduction of the *RAD52* gene product. Alternatively, yeast may have evolved a more efficient mechanism than phage T4 for recognizing and repairing UV-induced lesions, which depends on the *RAD52* gene product.

Besides the similarity between phage T4 genes *46* and *47* and yeast gene *RAD52*, there are a number of other interesting similarities between genes of phage T4 and genes of eukaryotes or their viruses. The type II topoisomerase subunit of phage T4 encoded by gene *52* shows significant homology with the carboxyl half of yeast DNA topoisomerase II (51). The phage T4 DNA polymerase encoded by gene *43* shares regions of sequence similarity with human DNA polymerase α, and the DNA polymerases of herpes family viruses, vaccinia virus, and adenovirus (52). The phage T4 thymidylate synthetase also shows considerable homology with yeast thymidylate synthetase (53). The DNA ligase of phage T4 and the ligases of both budding and fission yeast

show sequence similarity over the specific stretch of amino acids that form the putative ATP-binding region (54, 55). Introns have been observed in genes *td* (56), *nrd*B (57), and *ORF55* (58) of phage T4, which encode thymidylate synthetase, nucleotide reductase small subunit, and an unidentified gene product, respectively. The *td* intron shares homology with three self-splicing group I introns of mitochondrial genes of filamentous fungi (59). The above similarities between genes and introns of phage T4 and those of eukaryotes indicate that these entities arose in a common progenitor. That they have retained common genetic information in their separate lineages implies selective pressure to retain an early beneficial adaptive structure. Therefore, any knowledge gained about gene products, such as gene products *46* and *47*, in the relatively accessible phage T4 system may be pertinent to the homologous gene products in eukaryotes.

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