The Product of the Split *sunY* Gene of Bacteriophage T4 Is a Processed Protein

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The *sunY* gene of bacteriophage T4 contains a self-splicing group I intron. The ligated exons encode an open reading frame of 605 amino acids, whose inferred molecular mass is 68 kDa. However, none of the proteins made following T4 infection have been assigned to the *sunY* gene, and no mutations have been mapped to this locus. We show here that the primary product of the *sunY* gene is a protein with an apparent molecular mass of 64 kDa, which is processed to a protein approximately 4 kDa smaller. Unlike most other processed T4 proteins, cleavage occurs independently of both the T4 processing protease, the product of gene 21, and late phage protein synthesis. Insertional mutagenesis demonstrated that the *sunY* protein is not necessary for normal T4 growth under the conditions tested.

The T4 *sunY* gene is one of four bacteriophage genes that have been identified as containing self-splicing group I introns (7, 12, 30, 32). The functions of three of the four intron-containing phage genes are known. The *td* gene (encoding thymidylate synthase) and the *nrdB* gene (encoding the small subunit of ribonucleoside diphosphate reductase) of phage T4 are both involved in synthesis of DNA precursors, and the intron identified in phage SPO1 is in gene 31, encoding DNA polymerase. The function of the *sunY* gene, however, is unknown.

The *sunY* gene is not essential, as far as we know, since mutations that interfere with phage development have not been mapped to this locus. As a first step in characterizing the function of the *sunY* gene, we identified the *sunY* protein and determined the time course of its synthesis. Although we have not yet determined a function for the *sunY* gene, we made the curious observation that its expression involves processing of the protein as well as the RNA gene products (gp).

MATERIALS AND METHODS

Strains and plasmids. *Escherichia coli* B_E (*sup*⁰) (3) and T4 deletion phages SaΔ9 and rNB2226 were provided by L. Gold. SaΔ9 contains a 2.4-kb deletion in the D region extending from *ac* through D1 (9), while rNB2226 contains an approximately 6-kb deletion spanning the *rII* region (1). *E. coli* XA101 [*F*[−] *ara* Δ(*lac-pro*) *nalA supD metB argE*(Am) *rif thi*] was from E. Eisenstadt. T4 *alt* (*ac-q*) (15) phage were from C. Goff, and amber mutants in T4 genes 21 (*amH11*) and 23 (*amE322-amN121*) were from the collection of L. Black. J. Karam provided T4 gene 55 amber mutant *amBL292* (3). T4 deletions *farP13*, *farP23*, *tk2*, and *tk25* (6) were from D. Hall, and deletion Δ9A (21), isolated by D. Parma, was from J. Wiberg.

Descriptions of all relevant plasmids are given in Table 1. The *sunY* gene was inactivated by insertional mutagenesis in plasmid pAHZ2, which was created by inserting a T4 *rIIB-E. coli lacZ* gene fusion cassette into a *PvuII* site in the *sunY*

intron (see Fig. 1). The *rIIB-lacZ* gene fusion of pAHZ2 was transferred to the *sunY* gene of T4 rNB2226 by homologous recombination during infection of *E. coli* XA101 harboring the plasmid. The new phage was designated rNB2226, *sunY*[*rIIBlacAHZ2*].

A deletion within the *sunY* gene was created in plasmid pΔX3 (Table 1). The 1,292-bp deletion in this plasmid extends from position 2747 in exon 1 of the *sunY* gene to position 4039 in exon 2 (34). The resultant in-frame deletion spanning the intron removes codons 172 to 257 from the *sunY* exons and adds one extra codon (UCU) during the construction. The new gene product should be 85 amino acids shorter than *gpsunY*.

The *sunY* deletion was introduced into phage, with or without a gene 55 amber mutation (*amBL292*), by recombination between plasmid DNA bearing the *sunY* deletion and homologous sequences in the phage. The recipient phage used in this experiment contained a fusion of the *sunY* intron open reading frame (ORF) with *lacZ* (13). Recombinant phage (containing the deletion) were identified by loss of β-galactosidase and were designated *sunY*Δ3 or 55*am, sunY*Δ3.

Transfer of plasmid sequences to T4. Plasmid-containing strains of *E. coli* were grown in M9S medium (3) supplemented with 50 μg of ampicillin per ml to a density of 2 × 10⁸ cells per ml and infected at a multiplicity of 1. After 5 min at 30°C, the cultures were diluted 100-fold into M9S medium and incubated for 1 to 2 h. Chloroform was added to lyse cells remaining in the culture. The progeny were plated and screened for their ability to form blue plaques on plates containing 0.04% 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) with a *lac* deletion lawn (*E. coli* XA101) (or for the loss of the ability to form blue plaques in the case of the *sunY* internal deletion phage).

Labeling of cell- and phage-encoded proteins. Cells were grown at 30°C in M9 medium (3) supplemented with tryptophan (10 μg/ml), proline (25 μg/ml), thiamine (5 μg/ml), and ampicillin (50 μg/ml) (for plasmid-containing strains only) to a density of 2 × 10⁸ cells per ml. To label phage proteins, cells were infected at a multiplicity of approximately 5 to 7 and cultures (5 ml) were labeled for 2 or 5 min with [³⁵S]methionine (1,100 Ci/mmol) at 2.5 to 5 μCi/ml. To chase, nonradioactive methionine was added to 0.2 mg/ml.

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TABLE 1. Plasmid constructions

Plasmid	Relevant characteristics
pNC85	Derivative of pBR322 containing a deletion from positions 651 to about 2200 (33) and which also lacks the <i>Pst</i> I, <i>Hinc</i> II, <i>Bam</i> HI, and <i>Nde</i> I sites.
pNC82	pNC85 with an 873-bp <i>Hind</i> III fragment of the T4 <i>rII</i> genes (22) inserted at the <i>Hind</i> III site in the same orientation as the β -lactamase gene. Contains a unique <i>Hinc</i> II site in the <i>rIIB</i> gene.
pNC132	pNC85 lacking the <i>Acc</i> I site at position 2246 (33).
pAH100	pNC85 with a 785-bp <i>Hind</i> III fragment of the T4 <i>sunY</i> gene (positions 3179 to 3946) (34) inserted at the <i>Hind</i> III site in the opposite orientation from the β -lactamase gene.
pMC1871	pBR322 derivative containing the <i>lacZ</i> coding region flanked by polylinker sequences (26).
pSSZ1	pNC82 with the <i>Sal</i> I (filled-in)- <i>Sma</i> I <i>lacZ</i> -containing fragment of pMC1871 inserted into the <i>Hinc</i> II site to create an in-frame <i>rIIB-lacZ</i> gene fusion.
pAHZ2	pAH100 with the 3.9-kb <i>Hind</i> III fragment (ends were filled in with Klenow fragment of <i>E. coli</i> DNA polymerase I) of pSSZ1 (containing an <i>rIIB-lacZ</i> gene fusion) inserted into the <i>Pvu</i> II site in the <i>sunY</i> gene (position 3550) (34).
pASE16	pNC132 with a 3.5-kb <i>Sph</i> I- <i>Eco</i> RV fragment of T4 DNA (positions 1956 to 5470) (34) spanning the entire <i>sunY</i> gene inserted between the plasmid <i>Sph</i> I and <i>Eco</i> RV sites.
pASXE16	pASE16 with a 7-bp <i>Xba</i> I linker (5'-CTCTAGA-3') inserted into a filled-in <i>Acc</i> I site (position 2747) (34) of the <i>sunY</i> gene.
pAAX3	pASXE8 with the 1,292-bp <i>Xba</i> I fragment internal to the <i>sunY</i> gene eliminated by digestion and religation.

After labeling, each sample was centrifuged for 2 min and resuspended in 0.1 ml of sodium dodecyl sulfate (SDS) sample buffer (10% glycerol, 0.64 M β -mercaptoethanol, 3% SDS, 0.065 M Tris-HCl [pH 6.8]). Incorporated radioactivity was measured by 5% trichloroacetic acid precipitation of aliquots of the resuspended samples. In all cases, counts incorporated remained constant or showed only a slight decrease in the chase samples. All samples were run on 8% polyacrylamide-0.1% SDS gels with a stacking layer (18). Molecular weights were determined by using a linear regression program. Protein standards used to calculate molecular weight were commercially prepared (Sigma high-molecular-weight protein standards) or were internal markers of T4 proteins that fell in the same region of the gel as the *sunY* proteins (gp46, 63.5 kDa; gp39, 58.5 kDa; gp23, 56 kDa) and whose molecular weights were derived from DNA sequences (20).

RESULTS

Identification of the *sunY* protein product. To identify the product of the *sunY* gene, we performed insertional mutagenesis (using an *rIIB-lacZ* fusion cassette) at various positions in the *sunY* gene. The insertions in *sunY* sequences were created in plasmids and transferred to phage by homologous recombination with the *sunY* sequences (5, 19). The recipient phage carried a deletion to ensure viability of phage containing extra DNA (29, 31). The deletion used in this experiment (*rNB2226*) spanned the *rII* genes, eliminating homology with the plasmid *rII* sequences.

One example of a plasmid containing an *rIIB-lacZ* inser-

FIG. 1. Plasmid used for insertional mutagenesis of the phage T4 *sunY* gene. Plasmid pAHZ2 contains an in-frame fusion between the T4 *rIIB* and *E. coli lacZ* genes inserted into the *sunY* intron at the *Pvu*II site at position 3550 (34). Numbers in parentheses refer to pBR322 coordinates (33), and the double curved lines indicate the position of a deletion of about 1,550 bp (5). *sunY* sequences are indicated by solid boxes, *rII* sequences by hatched boxes, and *lacZ* sequences by an open box. The β -lactamase gene (*Ap*^r) is indicated by a shaded box. Restriction sites in parentheses were eliminated during cloning. Direction of transcription is indicated by arrows. A and B refer to fragments of the *rII* genes, which are shown divided by a wavy line; ori, origin of replication. Distances are not drawn to scale.

tion in the *sunY* gene is shown in Fig. 1. When this construct was transferred to T4 carrying deletion *rNB2226*, phage containing the insertion produced a functional β -galactosidase fusion product and were easily identified as producing blue plaques on X-Gal plates. The most complete set of data is available for the insertion shown in Fig. 1, and all data on insertions presented here will refer to this construction. However, in addition to this insertion in the intron ORF, a number of different phage insertion mutants were constructed in exon 1, the intron core, and exon 2 of the *sunY* gene, and all produced essentially the same results (35).

When the protein products from phage containing the insertion were compared with those from phage containing a wild-type *sunY* gene, two proteins in the 60- to 70-kDa range were missing, one of which was presumably the product of the spliced *sunY* mRNA (Fig. 2) (gpsunY has a predicted size of 68 kDa [30]). It is unlikely that either protein was the product of the intron ORF of 258 codons, whose calculated molecular mass is 30 kDa (34). The slower-migrating protein was estimated to be approximately 64 kDa (an average from six determinations), while the faster-migrating protein was about 60 kDa. The size of the larger protein is in relatively good agreement with the predicted size of the spliced *sunY* gene product.

In addition, autoradiograms from phage containing the inserts of the *lac* cassette contained three new bands. One of these bands corresponded to the product of the *rIIB-lacZ* gene (approximately 120 kDa), and a second, minor band corresponded to a protein of around 90 kDa that is probably derived from β -galactosidase (17). The third new band corresponded to a protein of about 20 kDa, the expected size (20,212 Da from sequence data) of the exon 1 product of the unspliced *sunY* gene (34). Absence of the 60- to 70-kDa protein(s) and appearance of the 20-kDa exon 1 product

FIG. 2. Initial identification of the *sunY* protein. *E. coli* B_E was infected with either T4 rNB2226,*sunY*[rIIBlacAHZ2], containing a disrupted *sunY* gene (lane 1, see also Fig. 1), or T4 rNB2226, containing an intact *sunY* gene (lane 2), and the proteins were labeled from 6 to 11 min after infection with [³⁵S]methionine. Positions of the proteins eliminated by the insertion (▲), the β-galactosidase-related proteins (Δ), and the putative exon 1 product (○) are indicated. Molecular weight markers (×10³) visualized by Coomassie blue staining are shown on the right.

suggest that insertion of the *rIIB-lacZ* fusion gene in the intron ORF blocks splicing in vivo.

Pulse-labeling of cells at various times after infection showed that the putative *sunY* product first appeared between 6 and 11 min and that its rate of synthesis reached a maximum between 11 and 16 min (35). These results are consistent with the time course of *sunY* expression obtained previously from monitoring enzyme activity of a translational fusion between exon 1 of *sunY* and the *lacZ* gene (13).

Evidence for protein processing. The absence of two proteins from the *sunY* insertion mutant raised the question of whether the proteins were related as precursor and product or whether they were two completely independent proteins (for example, the insertion might be polar on downstream genes). There are several precedents for proteolytic processing of T4 proteins (16). For example, the products of genes 22, 23, 24, 67, IPI, IPII, IPIII, and *alt* are all proteolytically cleaved, and processed forms are incorporated into the capsid. The processing events are accomplished through the action of gp21 (known as the T4 processing protease), which is itself probably autocatalytically cleaved (27, 28).

To determine whether the *sunY*-associated proteins were actually precursor and product, we performed a pulse-chase experiment. Cultures of *E. coli* B_E were infected with either phage that contained an intact *sunY* gene or phage that contained an *rIIB-lacZ* insertion in the *sunY* intron. The proteins were labeled from 11 to 16 min after infection with

FIG. 3. *sunY* protein is processed and is distinguishable from the *alt* protein. *E. coli* B_E was infected with T4 rNB2226, which contains both a wild-type *alt* gene and an intact *sunY* gene (A), and *alt* phage (B). Proteins were labeled with [³⁵S]methionine from 11 to 13 min after infection and chased for the times indicated on the bottom (in minutes). The fifth lane of panel A is an identical pulse-labeling performed with phage containing an insertion in the *sunY* intron that disrupts splicing (T4 rNB2226,*sunY*[rIIBlacAHZ2], labeled here as *sunY*, see Fig. 2). The last lane of panel B shows proteins labeled during a 5-min pulse of uninfected cells (lane u). Positions of the pertinent proteins in panels A and B are indicated as follows: ▲, *sunY* precursor; Δ, *sunY* product; ●, *alt* precursor; ○, *alt* product; ×, gene 23 precursor; ⊗, gene 23 product. The prominent band migrating above the position of *gpalt* precursor is *gpRIIA* and is absent in the *rII* deletion NB2226 (A). Molecular weight markers (×10³) are shown on either side.

[³⁵S]methionine and then chased for 5, 10, and 20 min with cold methionine (Fig. 3A). It can be seen that during the chase, the intensity of the slower-migrating protein band decreased, while the intensity of the faster-migrating band increased. These results indicate that the larger protein is converted into a smaller form by some undetermined mechanism.

The *sunY* protein can be distinguished from the *alt* protein. The product of the *alt* gene of T4 is a protein that functions to ADP-ribosylate one α-subunit of *E. coli* RNA polymerase (10, 11, 24, 25). The primary translation product of the *alt* gene (calculated molecular mass of 76.8 kDa [14]) is proteolytically cleaved during maturation of the phage head (15). Since the putative *sunY* product is similar in size to the *alt* protein, and both are processed, it is possible that the *sunY* mutations prevent accumulation of *gpalt* and that the proteins missing in Fig. 2 are actually the *gpalt*. Figure 3B shows the results of an experiment in which *E. coli* B_E was infected with T4 *alt* phage, labeled from 11 to 16 min with [³⁵S]methionine, and then chased for up to 20 min. It is clear from their different positions in the gel that the putative *sunY* proteins are distinct from the *alt* proteins.

We confirmed the large change in apparent molecular weight originally reported (15) for *gpalt* (Fig. 3). However, Hilse and coworkers (14) have determined that the amino terminus of the mature *alt* protein is just seven amino acids from the presumed translation start. It is possible that there is a second cleavage site near the carboxyl terminus of the protein that would account for the observed change in apparent size.

FIG. 4. Expression and processing of the *sunY* protein in gene 21, gene 23, and gene 55 amber mutants. (A) *E. coli* B_E (*sup*⁰) was infected with gene 21 or gene 23 amber mutant phage, and proteins were labeled from 11 to 13 min with [³⁵S]methionine and chased for the times indicated (minutes) at the bottom of the gel. The first four lanes show proteins labeled in cells infected with a gene 21 amber mutant, and the next four lanes show proteins labeled in cells infected with a gene 23 amber mutant. The last lane (lane u) shows proteins labeled in uninfected cells. The locations of the *sunY* proteins are indicated with a solid triangle (precursor) and an open triangle (product). The position of gp23 precursor is marked (×). Molecular size standards (in kilodaltons) visualized by Coomassie blue staining are shown on the left. (B) In a separate experiment, *E. coli* B_E was infected with gene 55 amber mutant phage and the proteins were labeled as in panel A and chased for the times indicated (minutes). The last lane (lane u) represents proteins labeled in uninfected cells. The positions of the *sunY* proteins are indicated as in panel A. Molecular size standards (in kilodaltons) are shown on the right.

***sunY* protein processing occurs independently of genes 21, 23, and 55.** To determine whether the *sunY* protein is cleaved by the gene 21 processing protease, we examined the fate of *sunY* protein in a phage that is deficient in gene 21 function. *E. coli* B_E (*sup*⁰) was infected with a gene 21 amber mutant phage, proteins were labeled from 11 to 16 min with [³⁵S]methionine, and samples were withdrawn during a 20-min chase (Fig. 4A). For comparison, a similar infection was done using a gene 23 amber mutant. It is clear that in the gene 21 amber mutant, proteins such as gp23 (normally cleaved by the processing protease) are not processed but rather accumulate as precursors (for normal processing, see Fig. 3A), while the *sunY* protein is apparently processed normally.

In a similar experiment with a T4 gene 55 amber mutant (gene 55 encodes the RNA polymerase sigma factor required for transcription from late T4 promoters), processing of the *sunY* protein was again shown to be unaffected (Fig. 4B), which shows that processing is not dependent on any late T4 protein. This is in contrast to synthesis of the protein product of the *sunY* intron ORF, which is completely dependent on a functional gene 55 (13).

Internal deletion in the *sunY* gene. Conclusive evidence for the identity of the product of the *sunY* gene could be obtained if a deletion in the *sunY* ORF resulted in a corresponding decrease in size of the putative *sunY* protein. To accomplish this, we created a deletion that eliminated the 3' end of exon 1, the entire intron, and the 5' end of exon 2 of *sunY* (a total of 1,292 nucleotides, 85 codons of exon sequence) and resulted in an in-frame protein fusion between the remaining portions of exon 1 and exon 2.

The internal deletion was established in phage that were either gene 55 positive or gene 55 negative. The gene 55 mutant background resulted in a much simpler protein profile on gels, which aided in detection of the *sunY* proteins.

Phage containing the internal deletion no longer made the

normal *sunY*-associated proteins but did produce a new, smaller protein of approximately 54 kDa. This protein was located in an uncrowded region of the gel and comigrated with a protein that was barely detectable in 55am phage (Fig. 5C and D). The deletion product was not processed, suggesting that proper folding of the protein is required for processing. The residual exon sequences predict a size of 58 kDa, close to the size predicted by the mobility of the new protein.

A T4 deletion mutant that does not make the *sunY* protein. In an effort to identify genes involved in the production or processing of the *sunY* protein, we examined a number of T4 mutants that contained deletions of large portions of the T4 genome between genes 49 and *e* (6). None of these deletions overlap the *sunY* gene. An unmapped deletion mutant (Δ 9A) that is defective in synthesis of an unidentified 70-kDa early protein (21) was also examined to determine whether the missing protein was the *sunY* product.

Cultures of *E. coli* B_E were infected by each of the deletion phage, and the proteins were labeled with [³⁵S]methionine from 11 to 13 min and chased for 10 min. Results (not shown) indicated that while three of the five deletion phage, *farP23*, *tk2*, and *tk25* (6), made and processed the *sunY* protein, deletion phage Δ 9A and *farP13* (6) did not (although phage *farP13* did make an extra, smaller protein of undetermined origin). Hybridization experiments using various nick-translated plasmids (containing cloned portions of the T4 genome in the region of *sunY*) to probe plaques of each of the deletion phage suggest that at least part of the Δ 9A deletion falls in exon 2 of *sunY* (35).

Of the four deletions *tk2*, *tk25*, *farP23*, and *farP13*, the deletion in phage *farP13* is the largest and includes approximately 2.5 kb that does not overlap the other deletions. It was possible that a regulatory gene for *sunY* fell in this region uniquely deleted in *farP13* and that the absence of this gene resulted in lack of *sunY* gene expression. However, this

FIG. 5. Internal deletion in the *sunY* gene results in production of a smaller protein. Phage containing an internal deletion in the *sunY* gene along with a gene 55 amber mutation (D and F, 55*am*-, *sunY*Δ3) were used to infect *E. coli* B_E, and the proteins were labeled from 11 to 13 min after infection and chased as indicated. (D and F represent two separate isolates of the same phage.) As controls, B_E was also infected with a phage containing the gene 55 mutation alone (A, 55*am*), a disruption in the *sunY* gene (13) in the absence or the presence of 55*am* (B and C, SaΔ9*sunY*[AZP1] and 55*am*, SaΔ9*sunY*[AZP1], respectively), or the internal deletion (E, *sunY*Δ3). All phage except the gene 55 amber mutant alone were in an SaΔ9 background. The last lane (lane U) shows proteins labeled in uninfected cells. The positions of the *sunY* proteins are indicated with a solid triangle (precursor) or an open triangle (product). The position of the smaller *sunY* protein made as a result of the deletion is indicated with a hatched triangle. Molecular size standards were visualized by Coomassie blue staining and are shown on the right.

possibility was excluded by the observation that the *sunY* defect in *farP13* could be corrected by recombination with a plasmid containing wild-type *sunY* sequences (35). This indicates that lack of *sunY* protein production in the *farP13* phage is probably the result of a secondary mutation in the *sunY* gene. Backcrossing not only restored *sunY* production but also eliminated the small extra protein made by *farP13* phage, suggesting that this protein was a truncated version of the *sunY* product. These results suggest that the approximately 20 kb of T4 DNA included in the deletions is not involved in production or processing of the *sunY* protein.

DISCUSSION

This work identified *gpsunY* as a protein with an apparent molecular mass of 64 kDa that is processed during phage infection to a product approximately 4 kDa smaller. The size of the precursor protein is in good agreement with the predicted size (68 kDa) of the product of the *sunY* gene. Unlike a number of other well-characterized processed proteins in phage T4, processing of *gpsunY* is independent of gene 21, which encodes the T4 processing protease. Processing of other early proteins, such as IPI, IPII, and IPIII (2) and *gpalt* (15), has been reported but depends on late protein synthesis, presumably because the proteins are cleaved during capsid formation. However, the amino terminus of purified IPI precursor is actually codon 16 of the IPI ORF in T4 DNA (4), suggesting a prior gp21-independent cleavage between serine 15 and threonine 16. The *sunY* protein would then be another example of an early protein that is processed

independently of the synthesis of late proteins and capsid formation.

The *sunY* product is initially processed very rapidly, as evidenced by the observation of both precursor and product after only a 2-min pulse with [³⁵S]methionine, yet processing is not complete by 20 min (Fig. 3). This is in contrast to the gp21-dependent processing of gp23, that is almost complete in a 5-min chase (Fig. 3). One explanation for incomplete processing of the *sunY* protein is that a fraction of the protein may be associated with other proteins, rendering it processing resistant. Alternatively, it is possible that there is some factor required for *sunY* processing that is not available at later times in infection.

Several possibilities exist for the mechanism of the *sunY* processing event. In particular, we have not excluded phosphorylation or other posttranslational modification. If proteolysis is indeed involved, we do not know whether the protease used in *sunY* processing is of bacterial or phage origin. Indeed, it is possible that the molecule undergoes self-cleavage. Consistent with this hypothesis is the observation that the protein from *sunY*Δ3, with an internal deletion (Fig. 5), does not undergo processing. Since processing only reduces the molecular mass of the *sunY* protein by about 4 kDa, the deletion of codons 173 through 257 of the protein cannot have removed the cleavage site. However, if the *sunY* protein is self-cleaving, it cannot act in *trans* on the mutant protein, since a simultaneous infection of *E. coli* cells with wild-type and *sunY*Δ3 phage at multiplicities that ensured a high level of coinfection showed no processing of the deletion protein (35). Whatever the source of the protease, it seems that proper folding is essential for cleavage.

Insertional mutagenesis has shown that the *sunY* gene is nonessential under the laboratory conditions tested. These results suggest that either the *sunY* gene has no important function or the host is somehow providing a compensatory function. It is also possible that the *sunY* gene plays an essential role under different growth conditions, such as during anaerobiosis or growth in an alternative host, such as *Shigella dysenteriae* (23). Preliminary screening of the ability of *sunY* mutants to grow on a number of clinical isolates of *E. coli* that are known to restrict phage carrying mutations in other "nonessential" genes (8, 9) has failed to identify a host that is restrictive for *sunY* mutants.

The function of the *sunY* gene remains a mystery. The three other bacteriophage genes that contain intervening sequences are involved in DNA synthesis pathways. There is no evidence, however, to support a role for *sunY* in DNA synthesis. The occurrence of introns in phage genes is in itself intriguing when one considers the compact organization of the phage DNA. However, in the *sunY* gene, not only is the mRNA spliced to remove an intron, the protein is also processed. We consider it unlikely that all these reactions evolved to produce a product that is not important for phage reproduction. We will continue to search for the function of the *sunY* gene, and we are hopeful that once this function is known, it may help to clarify why such an elaborate scheme may be required for *sunY* gene expression.

ACKNOWLEDGMENTS

We thank Lindsay Black for telling us about gp21-independent processing of IPI and Carole Keith for excellent preparation of the manuscript.

This work was supported by Public Health service grant GM37746 from the National Institutes of Health. A.Z. was supported in part by a predoctoral training grant from the U.S. Army Research Office.

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