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Accessibility
A Single-stranded DNA-binding Protein of Bacteriophage T7 Defective in DNA Annealing*

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The annealing of complementary strands of DNA is a vital step during the process of DNA replication, recombination, and repair. In bacteriophage T7-infected cells, the product of viral gene 2.5, a single-stranded DNA-binding protein, performs this function. We have identified a single amino acid residue in gene 2.5 protein, arginine 82, that is critical for its DNA annealing activity. Expression of gene 2.5 harboring this mutation does not complement the growth of a T7 bacteriophage lacking gene 2.5. Purified gene 2.5 protein-R82C binds single-stranded DNA with a greater affinity than the wild-type protein but does not mediate annealing of complementary strands of DNA. A carboxyl-terminal motif and are essential for coordination of leading and lagging strand DNA synthesis in vivo. Gene 2.5 protein-R82C condenses single-stranded M13 DNA in a manner similar to wild-type protein when viewed by electron microscopy.

Gene 2.5 protein is required for bacteriophage T7 growth (1). Gene 2.5 protein acts as a nonspecific single-stranded DNA (ssDNA)1-binding protein, binding ssDNA preferentially over double-stranded DNA (2). ssDNA binding proteins participate in multiple steps of DNA replication, recombination, and repair (1–13). Whereas gene 2.5 protein is functionally equivalent to Escherichia coli SSB protein and the bacteriophage T4 gene 23 protein, it lacks significant sequence homology to these proteins, and neither of these proteins can replace its function (1–13). Whereas gene 2.5 protein is functionally equivalent to Escherichia coli SSB protein, gene 2.5 protein-R82C, binds even more tightly to single-stranded DNA than does gene 2.5 protein-R82C, but it anneals homologous strands of DNA as well as does the wild-type protein. The altered protein forms dimers and interacts with T7 DNA polymerase comparable with the wild-type protein. Gene 2.5 protein-R82C condenses single-stranded M13 DNA in a manner similar to wild-type protein when viewed by electron microscopy.

In addition to binding ssDNA and physically interacting with T7 DNA polymerase, gene 2.5 protein also facilitates the annealing of complementary strands of DNA (10, 11, 16). Homologous DNA annealing is a vital activity during the process of DNA replication, recombination, and repair (17). A number of proteins have evolved to carry out this vital function, such as the RecA protein (18, 19) and members of the single strand annealing family that includes the E. coli RecT protein, the Redβ protein from bacteriophage λ, and the eukaryotic annealing protein Rad52 (17, 20–23). Unlike the RecA protein, the gene 2.5 protein does not require ATP (16), and it cannot mediate strand transfer (11, 16). Gene 2.5 protein bears some similarity to the RecT protein and its family members, proteins that also mediate DNA annealing in an ATP-independent fashion (17). Structurally, gene 2.5 protein differs from members of this family, which form multimeric ring structures in the presence and absence of ssDNA (24–26). Gene 2.5 protein, on the other hand, is a dimer in solution (2), and its three-dimensional structure resembles that of other ssDNA-binding proteins (27). Similar to T4 gene 32 protein and E. coli SSB protein, gene 2.5 protein features an oligonucleotide/oligosaccaride binding fold (Fig. 1) (27). Although both T4 gene 32 protein and E. coli SSB protein have been shown to mediate DNA annealing (28, 29), T7 gene 2.5 protein does so much more efficiently (16).

The biochemical basis of the efficient DNA annealing activity of gene 2.5 protein is unknown. It seems likely that it involves interactions between two gene 2.5 protein-coated ssDNA molecules. A previous study has shown that the ability to bind ssDNA is critical for this reaction to occur (30). It is also possible that interactions at the dimer interface are involved in this process. Two gene 2.5 proteins with alterations in the dimer interface retained the ability to mediate DNA annealing, in a manner similar to the WT protein, whereas a third did so in a slightly longer time period (31). We have recently employed a genetic screen to identify functional domains in gene 2.5 protein (31). One of the alterations uncovered by the screen mapped to a loop connecting the prominent α-helix to the β-barrel portion of the structure (Fig. 1). The exact residue, Arg-82, resides in a disordered region of the structure. Here we describe the purification and characterization of this protein and show that it is defective in DNA annealing.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Phage—E. coli HMS838 (F’ hsdR pro leu-lac– thi– supE tonA– trcA–) and E. coli HMS 89 (xylB thi– argE metE xyl– str– R are’ his’ galK lacY proA leu– thr– tss– supE) were used as hosts for phage experiments. E. coli BL21 (DE3) (F’ ompT hsdSB(rB- mB-)gal– dem– (DE3)) (Novagen) was used to express gene 2.5. T7/2.5 phage used in the in vivo DNA synthesis experiments was provided by Jaya Kumar (Harvard Medical School).

Plasmids, Oligonucleotides, and Proteins—The following oligonucleotides were purchased from Oligos Etc: T7.25NdeI (5’-CGTAAA- TCCATATGGCTAAGAAGATTTTCACCTC-3’) and T7.25BamH1 (5’-CGT-
using the calculated extinction coefficient at 280 nm of 2.59 mM Tris-HCl (pH 7.5), 2 mM EDTA, 2% SDS was added to the sample. ° and after a 90-s incubation at 30°C (27) is shown with the liquid scintillation counting. 

**Protein Purification** Gene 2.5 protein-R82C was overproduced and purified using a procedure previously employed to purify WT gene 2.5 protein [2.5] based on the fact that expression of the mutated gene in DNA synthesis in vitro otherwise, reactions were incubated at 30°C with 10 μM gene 2.5 protein, and the reaction was stopped by the addition of SDS to a final concentration of 0.5%. Reaction products were analyzed on a 0.8% agarose gel at 75 V for 1 h at room temperature, dried under vacuum, and exposed to a Fuji phosphor imager plate, and radioactivity was calculated using ImageQuant software. Plots of the data represent the background-corrected average of three experiments.

**Electron Microscopy**—WT and altered gene 2.5 proteins or E. coli SSB protein were diluted to 500 ng/μl in 20 mM Hepes/NaOH, (pH 7.5), 20% glycerol, mixed with single-stranded WT M13 DNA at 25 ng/μl in a buffer containing 10 mM Hepes/NaOH (pH 7.5), 50 mM NaCl final concentration. MgCl2 was added to the reaction buffer to 10 mM where indicated. Binding reactions with protein/DNA ratios (μg/μg) ranging from 40:1 for WT gene 2.5 protein to 10:1 for mutants and E. coli SSB protein were incubated for 15 min at room temperature in a 50-μl total reaction volume.

Following the binding reactions, the samples were fixed with an equal volume of 1.2% glutaraldehyde for 5 min at room temperature and then loaded onto a 2-ml column of Bio-Gel A-5m previously equilibrated in 10 mM Tris-HCl (pH 7.5), 0.5 mM EDTA. The same buffer was then used to elute the sample from the column and 250-μl fractions were collected. Aliquots of the protein-DNA containing fractions were mixed with a buffer containing spermidine (33) for 3 s and quickly applied to a mesh copper grid coated with a thin carbon film, glow-charged shortly before sample application. Following adsorption of the samples to the electron microscopy support for 1–2 min, the grids were subjected to a dehydration sequence in which the wash solutions was gently replaced by a serial increase in ethanol concentration to 100% and then air-dried. The samples were then rotary shadowcast with tungsten at 10−3 torr and examined in a Philips CM 12 TEM instrument at 40 kV. Micrographs, taken at ×46,000, were scanned using a Nikon LS-4500AF film scanner, and panels were arranged using Adobe Photoshop.

**DNA Annealing Assay**—The ability of WT gene 2.5 protein to mediate the annealing of homologous strands of DNA was assessed using an in vitro annealing assay developed by Tabor and Richardson [16]. A 310-nucleotide internally labeled ssDNA fragment was generated as described previously [16, 31]. DNA annealing was assayed in reactions containing 4 nCi 32P-labeled ssDNA fragment, 20 μM M13 mGP2-2 ssDNA, 20 mM Tris-Cl (pH 7.5), 1 mM dithiothreitol, 10 mM MgCl2, 50 mM NaCl, and various concentrations of gene 2.5 protein. Unless noted otherwise, reactions were incubated at 30°C for 8 min. Time course experiments were carried out at 30°C with 10 μM gene 2.5 protein, and the reaction was stopped by the addition of SDS to a final concentration of 0.5%. Reaction products were analyzed on a 0.8% agarose gel at 75 V for 1 h at room temperature, dried under vacuum, and exposed to a Fujix phosphor imager plate, and radioactivity was calculated using ImageQuant software. Plots of the data represent the background-corrected average of three experiments.

**RESULTS**

**Gene 2.5 Protein-R82C Cannot Support T7 DNA Synthesis or T7 Phage Growth**—Gene 2.5 is essential for the growth of bacteriophage T7 (1). In this study, we examine a mutation, leading to a single amino acid change, arginine 82 to cysteine, that was isolated as part of a screen for lethal mutations in gene 2.5 (31). As such, it was unable to support the growth of T7 phage lacking gene 2.5 (TT22.5) (Table I). Interestingly, this mutation does not affect the function of WT gene 2.5 protein based on the fact that expression of the mutated gene from a plasmid does not inhibit the growth of WT T7 phage (Table I).

Since gene 2.5 is an essential gene and its product is involved in DNA synthesis in *vivo*, we examined the ability of gene 2.5
protein-R82C to carry out DNA synthesis in vivo. E. coli cells expressing the WT or mutant gene 2.5 protein were grown to midlog phase and then infected with a T7 phage lacking gene 2.5. At specific time points, aliquots of cells were removed and mixed with radioactively labeled thymidine. After 90 s, the reactions were terminated. Results of such an experiment are shown in Fig. 2. DNA synthesis peaks ~30 min after infection in cells expressing WT gene 2.5. As a control, no DNA synthesis is observed in cells harboring gene 2.5 lacking the coding sequence for the carboxyl-terminal motif (gene 2.5 protein-R82C). Similarly, DNA synthesis declines soon after infection in cells expressing gene 2.5 protein-R82C. Therefore, it is likely that this mutant is lethal because it is defective in some aspect of DNA metabolism.

Gene 2.5 Protein-R82C Binds ssDNA—One of the primary attributes of gene 2.5 protein is its ability to bind ssDNA (2). In the current study, we assessed the ability of the altered gene 2.5 proteins to bind ssDNA using an electrophoretic mobility shift assay. Using this method, we previously calculated the dissociation constant (K_D) for WT gene 2.5 protein to be 2.6 × 10^{-6} M (31). As shown in Fig. 3, the mobility of a 70-mer oligonucleotide is retarded as increasing amounts of gene 2.5 protein-R82C are added. Gene 2.5 protein-R82C binds ssDNA with ~10-fold higher affinity than does the WT protein (K_D = 3.0 × 10^{-7} M). Thus, the amino acid alteration causes the protein to bind ssDNA with a higher affinity than WT gene 2.5 protein. Since gene 2.5 protein-R82C retains this vital function, we consider it unlikely that the alteration results in a misfolded protein.

Like other ssDNA binding proteins, WT gene 2.5 protein binds ssDNA with a much higher affinity than double-stranded DNA (2). We examined the binding of gene 2.5 protein-R82C to double-stranded DNA using the electrophoretic mobility shift assay. Gene 2.5 protein-R82C bound a double-stranded 70-base pair DNA weakly and in a manner similar to the WT protein (data not shown). Thus, whereas the alteration, arginine 82 to cysteine, conferred higher ssDNA-binding affinity upon gene 2.5 protein, it did not lead to increased double-stranded DNA binding activity.

Gene 2.5 Protein-R82C Is Defective in DNA Annealing—Gene 2.5 protein can anneal homologous strands of ssDNA in vitro (16, 30, 31). In this study, we looked at the ability of WT and altered gene 2.5 proteins to anneal a 310-nucleotide ssDNA fragment to single-stranded M13 DNA. As previously shown (16), WT gene 2.5 protein can efficiently anneal these homologous strands of DNA (Fig. 4A). In this reaction, an internally labeled 310-nucleotide ssDNA is mixed with M13 circular ssDNA in the presence of varying concentrations of gene 2.5 protein. The labeled DNA fragment is homologous to a region of the M13 ssDNA. Annealing of the 310-nucleotide fragment to the homologous region of M13 ssDNA does not occur after an 8-min incubation at 30°C in the absence of gene 2.5 protein (Fig. 4A, lane 1), since we observe a single, rapidly migrating radioactively labeled species on an agarose gel. When the concentration of gene 2.5 protein in the reaction is increased, annealing of the DNA strands begins to occur. In Fig. 4A, lane 4, we observe two species, the faster migrating corresponding to the unannealed 310-nucleotide fragment and a more slowly migrating species corresponding to the annealed product. The more slowly migrating species is present even after extraction with phenol chloroform (data not shown), suggesting that the gel shift is due to the increase in size of the annealed product and not a function of gene 2.5 protein binding to the ssDNA. At even higher concentrations (Fig. 4A), all of the labeled fragment is annealed to the M13 circular ssDNA.

As previously shown (16), DNA annealing is not observed under the same conditions when E. coli SSB protein is added to the reaction (Fig. 4B). Instead, a third species that migrates faster than the annealed product and slower than the fragment is observed upon the addition of E. coli SSB protein. Such a gel shift is noted in all protein concentrations tested (Fig. 4B, lanes 2–7). This species migrates more rapidly than the annealed product produced by gene 2.5 protein under the same conditions (Fig. 4B, lane 8). At pH 7.5, DNA annealing by E. coli SSB protein is dependent on the presence of a polyamine (28). Since we did not include polyamine in our assay, it is not surprising that E. coli SSB protein could not mediate this reaction under the conditions employed in this study.
facilitate DNA annealing (11). Annealing occurred at a 10-
other carboxyl terminus-deleted protein, gene 2.5-
These results agree with previous studies that showed an-
protein (Fig. 4)
ing of ssDNA at even lower concentrations than does the WT
H9004

Gene 2.5 protein-R82C is defective in DNA annealing (Fig.
C. At the highest concentration test (45 μM), only ~25% of the
fragment is converted to annealed product (Fig. 4C, lane 6).
Under the same conditions, WT gene 2.5 protein anneals 100%
the fragment at a concentration of 15 μM (Fig. 4A, lane 5).
Like E. coli SSB protein, gene 2.5 protein-R82C has a higher
affinity for ssDNA than the WT protein. Thus, it is not surpris-
ing that we observe the appearance of a band that probably
corresponds to a protein-DNA complex as the concentration of
gene 2.5 protein-R82C in the reaction is increased (Fig. 4C,
lanes 2–6). Next, we compared DNA annealing mediated by the
WT protein with annealing mediated by gene 2.5 protein-R82C
over a 4-min time period. In Fig. 5, we show that the WT
protein anneals nearly all of the labeled fragment in the reac-
tion in less than 3 min. In contrast, when the same concentra-
tion of gene 2.5 protein-R82C is added to the reaction, no
annealed product is observed over the 4-min time course.

Gene 2.5 protein-R82C and E. coli SSB protein both have a
higher affinity for ssDNA than WT gene 2.5 protein (10- and
50-fold, respectively). In addition, they are both defective in
annealing homologous strands of DNA under conditions that
are optimal for the WT gene 2.5 protein annealing activity.
We asked if these two properties were related. One model
might be that the increased DNA affinity impedes the disso-
ciation of the protein from ssDNA, which would be required
to complete the annealing reaction. To test this hypothesis,
we examined the ability of another altered gene 2.5 protein,
gene 2.5 protein-Δ26C, to facilitate annealing. Previously, we
showed that gene 2.5 protein-Δ26C has a higher affinity for
ssDNA than does the WT protein, with a dissociation con-
stant of 3.6 × 10⁻⁸ M (30). This protein facilitates the anneal-
ing of ssDNA at even lower concentrations than does the WT
protein (Fig. 4D) and does so at a slightly higher rate (Fig. 5).
These results agree with previous studies that showed an-
other carboxyl terminus-deleted protein, gene 2.5-A21C, can
facilitate DNA annealing (11).² Annealing occurred at a 10-
fold lower concentration than it did in reactions with WT
gene 2.5 protein, demonstrating that the higher affinity for
ssDNA did not lead to defective annealing. Therefore, other
explanations for the defect in annealing exhibited by gene 2.5
protein-R82C must pertain.

Gene 2.5 Protein-R82C Condenses ssDNA—WT gene 2.5 protein condenses circular M13 ssDNA in the presence of magne-
sium (2), resulting in compact structures when viewed by elec-
tron microscopy. The M13 circular ssDNA molecule appears as
a collapsed structure when viewed by electron microscopy (Fig.
6A, in contrast to the open configuration observed with E. coli
SSB protein (Fig. 6B). Unlike WT gene 2.5 protein, E. coli SSB
protein binds DNA and opens the circle both in the presence

² S. Tabor and C. C. Richardson, unpublished observation.
and absence of magnesium (Fig. 6, compare B and C with D and G). Since E. coli SSB protein does not facilitate DNA annealing under the same conditions as does gene 2.5 protein, it is conceivable that the annealing activity is related to the ability of gene 2.5 protein to condense M13 ssDNA. Therefore, we examined interaction between the DNA annealing-defective protein, gene 2.5 protein-R82C, and M13 ssDNA using electron microscopy. Gene 2.5 protein-R82C generates a structure with M13 ssDNA similar to WT gene 2.5 protein. The M13 circle appears in an open form in the absence of magnesium and as a condensed structure in the presence of magnesium (Fig. 6, E and H). It also appears that more gene 2.5 protein-R82C is bound to M13 DNA than the WT protein, most likely the consequence of the higher affinity gene 2.5 protein-R82C has for ssDNA. We examined another higher affinity variant of gene 2.5 protein, gene 2.5 protein-Δ26C. This variant also condenses ssDNA in the presence of magnesium (Fig. 6D). Interestingly, it does not readily form open structures in the absence of magnesium but rather collapses the ssDNA upon itself (Fig. 6F). Since both gene 2.5 protein-R82C, which cannot anneal homologous strands of DNA as efficiently as does the WT protein, and gene 2.5 protein-Δ26C, which does anneal homologous strands, appear to condense M13 ssDNA in the presence of magnesium, we conclude the two properties are not related.

**Gene 2.5 Protein-R82C Is a Dimer**—WT gene 2.5 protein is a dimer in solution (2). Using gel filtration analysis, we find that gene 2.5 protein-R82C elutes from the column at the same volume as does the WT gene 2.5 protein (Fig. 7). The column was calibrated, and a standard curve was generated by determining the elution volume of a series of molecular weight markers, specifically RNase A (13.7 kDa), chymotrypsinogen (25 kDa), ovalbumin (43 kDa), and bovine serum albumin (67 kDa). Using the standard curve, the molecular weight of gene 2.5 protein-R82C was estimated to be 58,200, which is consistent with the protein forming a dimer in solution. Thus, the altered proteins can form dimers, further suggesting that single amino acid substitution does not lead to misfolding of the protein.

**Gene 2.5 Protein-R82C Physically Interacts with T7 DNA**

**Fig. 6.** Electron microscopic analysis of gene 2.5 proteins binding to ssDNA. Electron micrographs of gene 2.5 proteins and E. coli SSB protein bound to M13 ssDNA. Samples were fixed with glutaraldehyde and further prepared for electron microscopy as described under “Experimental Procedures.” All photographs are at a × 46,000 magnification. Protein-free M13 DNA is shown (A) as well as E. coli SSB protein-bound M13 DNA (mass ratio of 10:1) in the absence (B) and presence (C) of 10 mM MgCl₂. WT gene 2.5 protein was incubated with M13 ssDNA at a mass ratio of 40:1 in the absence (D) and presence (G) of 10 mM MgCl₂, whereas reactions for gene 2.5 protein-Δ26C (E and H) and gene 2.5 protein-R82C (F and I) were carried at a 10:1 mass ratio to DNA, in the absence and presence of magnesium, respectively.

**Fig. 7.** Gene 2.5 protein-R82C is a dimer in solution. Gel filtration was carried out as described under “Experimental Procedures.” Gene 2.5 protein-R82C was loaded on a Sephadex-75 column in three independent experiments. A standard curve was generated by plotting Kₐ versus log Mᵣ for known molecular weight standards. The Kₐ for WT gene 2.5 protein, gene 2.5 protein-R82C, and gene 2.5 protein-Δ26C are noted along the curve. Data for WT gene 2.5 protein and gene 2.5 protein-Δ26C were published previously (31) and are shown here for comparison. The following standards were used in this experiment: albumin (67 kDa), ovalbumin (43 kDa), chymotrypsinogen A (25 kDa), and ribonuclease A (13.7 kDa).

**Polymerase**—Previous studies have shown that gene 2.5 protein physically and functionally interacts with T7 DNA polymerase (3), an interaction that requires the carboxyl terminus of the protein (14). We followed this interaction using surface plasmon resonance (31). In these experiments, we bound WT or altered gene 2.5 protein on a nickel-NTA coated chip and then passed T7 DNA polymerase over the bound protein. When the interaction was assessed in 100 mM NaCl, T7 DNA polymerase bound both WT gene 2.5 protein and gene 2.5 protein-R82C (Fig. 8A). The dissociation constant for these interactions was...
calculated to be $2.97 \times 10^{-6}$ and $1.28 \times 10^{-6}$ for the WT and altered protein, respectively. By contrast, T7 DNA polymerase does not bind gene 2.5 protein-Δ26C, which lacks the acidic carboxyl terminus (Fig. 8A). When the salt concentration was raised to 200 mM NaCl, T7 DNA polymerase did not bind to WT gene 2.5 protein or gene 2.5 protein-R82C (Fig. 8B). We conclude that gene 2.5 protein-R82C interacts with T7 DNA polymerase with approximately the same affinity as WT gene 2.5 protein.

**DISCUSSION**

Bacteriophage T7 gene 2.5 is an essential gene that encodes a single-stranded DNA binding protein (1). In addition to binding ssDNA, it can also mediate annealing of homologous strands of DNA (16). In the current study, we examined a lethal mutation in gene 2.5 that results in a single amino acid change in gene 2.5 protein, arginine to cysteine. The altered protein, gene 2.5 protein-R82C, is defective in DNA annealing. Gene 2.5 protein-R82C also binds ssDNA 10-fold more tightly than WT protein. This binding activity and pairing of gene 2.5 protein-bound DNA features of gene 2.5 protein are essential for annealing: ssDNA binding is required, since gene 2.5 protein-Δ26C displays the same preference for ssDNA as the WT protein. Thus, a defect in the second prop-

### Fig. 8. Gene 2.5 protein-R82C interacts with T7 DNA polymerase.

The interaction between gene 2.5 protein and T7 DNA polymerase was monitored using surface plasmon resonance on a BIAcore 3000. In all panels, the base line has been normalized to zero. Time(s) is plotted on the x axis; resonance units are plotted on the y axis. A, comparison of 500 nM T7 DNA polymerase binding to WT gene 2.5 protein, gene 2.5 protein-R82C, or gene 2.5 protein-Δ26C immobilized on an NTA chip charged with NiCl$_2$ in a buffer containing 100 mM NaCl. T7 DNA polymerase was passed over the chip and then allowed to dissociate for 10 min. B, comparison of 500 nM T7 DNA polymerase binding to WT gene 2.5, gene 2.5 protein-R82C, or gene 2.5 protein-Δ26C immobilized on an NTA chip charged with NiCl$_2$ in a buffer containing 200 mM NaCl. T7 DNA polymerase was allowed to flow over the chip and then allowed to dissociate for 10 min.
DNA Annealing by T7 ssDNA-binding Protein

entry, pairing of gene 2.5 protein when bound to ssDNA, seems more plausible. We initially hypothesized that the condensed structure of circular M13 ssDNA bound to gene 2.5 protein observed by electron microscopy (2) was correlated with this property. However, it appears that this phenomenon seems to be related to ssDNA binding, since gene 2.5 protein-R82C condenses ssDNA in a similar manner (Fig. 6). There are a number of structural elements that could be involved in the pairing of ssDNA-bound gene 2.5 protein. It is conceivable that the carboxyl-terminal domain is involved, since it has been shown to be important in interactions with other T7 DNA replication proteins (12, 14, 15). However, the carboxyl-terminal truncated version of gene 2.5 protein, gene 2.5 protein-Δ26C, anneals DNA (Fig. 4). The dimer interface might also be involved. However, a previous study has shown that two different gene 2.5 proteins with alterations in the dimer interface retain the ability to efficiently anneal DNA, whereas a third does so at a slightly slower rate (31). Whether gene 2.5 protein dimerizes when bound to DNA is still unknown. If it does so, then the structural motifs involved in that process may hold the key to the defect in gene 2.5 protein-R82C.

Recently, a three-dimensional structure of the DNA annealing domain of human Rad52 was solved (24, 25). The domain crystallized as an undecameric ring. Interestingly, the monomer does not have the same oligonucleotide/oligosaccharide binding fold that is found in gene 2.5 protein and other ssDNA-binding proteins. However, a structure-based alignment shows significant similarities between gene 2.5 protein and the annealing domain of human Rad52. The proposed DNA binding cleft of gene 2.5 protein consists of helix $\alpha_1$ and sheets $\beta_2$-$\beta_4$, $\beta_3$, and $\beta_4$ on one side and strands $\beta_5$ and $\beta_6$ on the other side (27). This cleft aligns quite well with a similar region on the hRad52 structure consisting of helix $\alpha_2$ and sheets $\beta_3$, $\beta_4$, and $\beta_5$ on one side and sheets $\beta_6$ and $\beta_7$ on the other. In addition, the aromatic residues tyrosine 111 and tyrosine 158, which are positionally conserved among ssDNA-binding proteins (27), are structurally conserved in hRad52 (tyrosine 65 and tyrosine 126, respectively). The conserved tyrosine residues from gene 2.5 protein form a trinucleotide binding motif that is also found in E. coli SSB protein and human RPA 70 (27). A variant of gene 2.5 protein where tyrosine 158 is changed to an alanine binds ssDNA with 10-fold lower affinity than the WT protein (30). Interestingly, these residues are also highly conserved in other eukaryotic Rad52 and Rad22 proteins (17, 24). Therefore, despite the lack of sequence homology and the general structure, there are a number of structural similarities between gene 2.5 protein and hRad52 that suggest functional homology, and it is conceivable that they work by similar mechanisms.

The single amino acid change described in this paper, arginine 82 to cysteine, is lethal to bacteriophage T7. The altered protein has two distinct differences when compared with the WT protein, increased ssDNA binding affinity and a defect in DNA annealing activity. Since gene 2.5 protein has multiple functions in bacteriophage T7 replication, it is difficult to pinpoint which of these changes is responsible for the lethal phenotype. The binding affinity of gene 2.5 protein-R82C is 10-fold higher than the WT protein, which could account for the lethal phenotype. However, we feel this is unlikely, since bacteriophage T7 grows in the host E. coli cells that express E. coli SSB protein, a nonspecific ssDNA-binding protein with an affinity for ssDNA higher than gene 2.5 protein (2). Whereas we cannot exclude the possibility that higher ssDNA binding affinity affects T7 growth, we feel it is more likely that the defect in DNA annealing accounts for the lethal phenotype.

If indeed the lethality is due solely to the defect in mediating homologous base pairing, then it would suggest that the annealing activity is essential for T7 survival. DNA annealing is important in both DNA repair and recombination. Bacteriophage T7 has a high rate of recombination, and mutations in gene 2.5 disrupt recombination (1, 26). Although it is not known whether recombination is essential for phage growth, it is likely that extreme breakage of the T7 chromosome without subsequent annealing to form recombinant molecules could be lethal. Homologous base pairing is also essential to one step in T7 DNA replication, the formation of concatamers. The bacteriophage T7 genome has a terminal redundancy of 160 nucleotides (39), which allows the ends to be replicated via concatemer formation (reviewed in Ref. 40). Annealing of these terminally redundant ends by gene 2.5 protein could be vital for concatemer formation and therefore for DNA replication. In fact, purified gene 2.5 protein is required to reconstitute T7 concatemer formation in vitro (41). Thus, it is likely that this is the reason that a plasmid expressing the annealing-defective gene 2.5 protein-R82C cannot complement the growth of bacteriophage T7Δ2.5.

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