Synergistic Action of Three Recombination Gene Products of Bacteriophage T4, uvsX, uvsY, and Gene 32 Proteins*

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Previously we proved that uvsX protein catalyzes reactions similar to those thought to occur during T4 recombination (Yonesaki, T., Ryo, Y., Minagawa, T., and Takahashi, H. (1985) Eur. J. Biochem. 148, 127–134; Yonesaki, T., and Minagawa, T. (1985) EMBO J. 4, 3321–3327). Now we have found that uvsY protein stabilizes binding of uvsX protein and single-stranded DNA and weakly stimulates those reactions catalyzed by uvsX protein, although it has little activity, if any, by itself. Gene 32 protein also stimulates them weakly at low concentrations, but is strongly inhibitory at high concentrations (Yonesaki, T., and Minagawa, T. (1985) EMBO J. 4, 3321–3327; Formosa, T., and Alberts, B. M. (1986) J. Biol. Chem. 261, 6107–6118). This inhibition is counteracted by uvsY protein. The highest efficiency of uvsX protein is achieved by the co-existence of a small amount of uvsY protein and a large amount of gene 32 protein. We discuss the mechanism and the role of the three proteins in recombination.

Current models of recombination propose that strand exchange initiates by pairing of ssDNA and homologous dsDNA (Meselson and Radding, 1975; Szostak et al., 1983). recA protein (Mr = 38,000) (Radding, 1982) of Escherichia coli and uvsX protein (Mr = 44,000) (Yonesaki et al., 1985; Yonesaki and Minagawa, 1985; Fujisawa et al., 1985; Formosa and Alberts, 1986) of bacteriophage T4, the key enzymes in this reaction, bind to ssDNA and promote pairing between the ssDNA and a homologous duplex molecule. The function of both key enzymes is enhanced by a class of single strand binding proteins such as SSB protein (Mr = 18,500) of E. coli and gene 32 protein (Mr = 33,500) of T4 phage within a limited range of the protein concentration (Shibata et al., 1979; Yonesaki and Minagawa, 1985). Probably at lower concentrations, the single strand binding proteins facilitate the binding of the key enzymes to ssDNA (Flory and Radding, 1982; Cox and Lehman, 1982; Yonesaki and Minagawa, 1985), while at higher concentrations they prevent the binding (Griﬃth et al., 1984; Yonesaki and Minagawa, 1985; Kowalczykowski and Krupp, 1987).

Mutants in uvsY gene, which also controls genetic recombination and repair of damaged DNA of T4 phage, present phenotypes similar to those of mutants in the uvsX gene (Cunningham and Berger, 1977; Yonesaki and Minagawa, 1987), suggesting that uvsY protein acts in concert with uvsX protein. The finding that cloned uvsY gene substantially enhanced the in vivo function of cloned uvsX gene (Minagawa et al., 1988) is consistent with this idea. Prompted by these facts, we tested whether the uvsY protein would behave as a constituent of an in vitro T4 recombination system, although our previous work with the partially puriﬁed uvsY protein revealed no activity relating to DNA recombination or stimulation of uvsX function (Yonesaki et al., 1985). Recently, we were able to produce large amounts of highly puriﬁed uvsY protein from the bacterial cells bearing a plasmid which overproduces the protein and chose to re-examine the function of the puriﬁed uvsY protein. The protein has no activity by itself except for weak activity in renaturation of complementary ssDNA, but binds to ssDNA and enhances the activity of uvsX protein even under unfavorable conditions in reactions relating to strand exchange. The highest efficiency of uvsX protein in the single strand assimilation reaction is attained when small amounts of uvsY protein and large amounts of gene 32 protein are also added, the latter being strongly inhibitory to the uvsX protein function if added alone.

MATERIALS AND METHODS

Puriﬁcation of Proteins—uvsX and uvsY proteins were extracted from the respective protein-overproducing cells constructed according to Fujisawa et al. (1985). Brieﬂy, a 3.4-kilobase pair EcoRI-cleaved DNA fragment containing uvsX gene or a 2.2-kilobase pair PartI-cleaved DNA fragment containing uvsY gene was cloned downstream from the λ phage promoter, P0, in a plasmid pNT45. Competent cells were co-transformed with either plasmid and pNT204 which codes for a λ repressor. Transformed cells were aerated at 30 °C to reach a cell density of about 4 × 10^8/ml, and then the protein synthesis was induced by heating at 42 °C for 1.5 to 3h. The proteins were extracted and puriﬁed by a modiﬁcation of the previously reported protocol (Yonesaki et al., 1985); a step condensing uvsX protein with a DIAFL0 membrane, and successive ﬂow-through steps for uvsY protein puriﬁcation through DEAE-cellulose and phosphocellulose columns in the presence of Triton X-100 were omitted. About 10 mg of uvsX protein (>95% purity) and about 5 mg of uvsY protein (>95% purity) were obtained from 1000 ml of culture. Gene 32 protein was puriﬁed according to the method described previously (Yonesaki and Minagawa, 1985).

Assays for Protein Activities—Assay conditions and methods for DNA binding, ATP hydrolysis, renaturation of complementary ssDNA, and single strand assimilation are described in each figure legend. Concentration of DNA is expressed in terms of nucleotides from calculations based on the fact that the average molecular weight of a nucleotide is 300.

Reagents—ATP was purchased from Pharmacia LKB Biotechnology Inc., ATP-γ-S from Boehringer, nitrocellulose ﬁlters from Sarturios, and agarose from Sigma. DNAs were prepared as described previously (Yonesaki and Minagawa, 1985).

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‡ The abbreviations used are: ssDNA, single-stranded DNA; ATP-γ-S, adenosine 5'-O-(thiotriphosphate); dsDNA, double-stranded DNA.
RESULTS

DNA-binding Activity of uvsY Protein—uvsY protein (M, = 16,000) shows affinity to both ssDNA and dsDNA when assayed by DNA-cellulose column chromatography (Yonesaki et al., 1985). To quantify this DNA-binding activity, we incubated various amounts of uvsY protein and 32P-labeled fd RFI (circular superhelical dsDNA) or 32P-labeled fd phage DNA (circular ssDNA) at 37°C for 10 min and assayed complex formation measuring 32P radioactivity retained after filtration through nitrocellulose membrane (Yonesaki and Minagawa, 1985). The results of this experiment shown in Fig. 1 indicate that (i) binding increases in a sigmoidal fashion as a function of uvsY protein concentrations, suggesting that the binding is cooperative, (ii) the protein binds better to ssDNA than to dsDNA, particularly at low concentrations of protein and DNA, and (iii) the protein binds stoichiometrically to ssDNA but not to dsDNA. The binding to dsDNA increases as a function of concentration of both protein and DNA. In contrast, binding to ssDNA is proportional to protein concentration, but independent of DNA concentration; binding reaches saturation when the ratio of ssDNA nucleotides to uvsY protein monomer approaches 14. We also examined binding of the protein to fd RFII (RFI linearized by a single cut with a restriction enzyme HpaI) and found no difference from RFI binding (data not shown). Further differences in the binding of uvsY protein to ssDNA and dsDNA are disclosed by the filter assay. First, 0.1 M NaCl does not affect the binding to ssDNA, but completely inhibits the binding to dsDNA. Second, the binding to ssDNA occurs rapidly and efficiently even at 0°C, whereas the binding to dsDNA is slow at 0°C (only 20–50% of the maximum binding is attained after more than 5 min) but rapid at 37°C (complete binding within 2 min). Finally, Mg2+ (7 mM) does not affect binding of the protein to ssDNA, but reduces that to dsDNA. From these observations, we conclude that uvsY protein has higher binding affinity for ssDNA than for dsDNA.

Complexes of uvsY protein with ssDNA and dsDNA were both stable at 37°C when uvsY protein was present in saturating amounts. After 20 min at 37°C, the fraction of the protein-bound DNA was more than 80% for ssDNA and 70% for dsDNA. Complexes of uvsY protein and DNA (dsDNA or ssDNA) were electrophoresed through 1% agarose gel at room temperature and visualized by staining with ethidium bromide. The complexes do not enter the gel when protein concentration is higher than saturating, but they do migrate into the gel, at various rates slower than free DNA, when protein concentration is low (data not shown).

Stabilization of uvsX Protein-ssDNA Complex by uvsY Protein—uvsX protein binds cooperatively to both ssDNA and dsDNA, resulting in the formation of nucleoprotein filament (Griffith and Formosa, 1985; Yonesaki and Minagawa, 1985). uvsX protein binds to ssDNA very efficiently at 0°C, and the complex is stable at this temperature, but dissociates rapidly when transferred to 30 or 37°C. In contrast, the complex with dsDNA is stable at these temperatures (Yonesaki and Minagawa, 1985). We have investigated the effects of uvsY protein on the stability of the uvsX protein-ssDNA complex in the presence of 1 mM ATP. The addition of ATP weakly raises the stability of binding of uvsX protein to ssDNA (Yonesaki and Minagawa, 1985). When we measured the stability of 32P-labeled fd DNA-uvsX protein complex as radioactivity retained on a membrane filter after being transferred from 0 to 37°C, we found that it decayed proportionally to time with a half-life of 4 min (Fig. 2). Thus, more uvsX protein is able to remain complexed with ssDNA during incubation at the physiological temperature through the action of uvsY protein. In this sense, uvsY protein confers stability to uvsX protein-ssDNA complex.

ATP Hydrolysis in the Presence of Three Recombination Proteins—Gene 32 protein binds cooperatively to ssDNA (Alberts and Frey, 1970; von Hippel et al., 1977; Griffith and Formosa, 1985) and facilitates single strand assimilation by uvsX protein (Yonesaki and Minagawa, 1985). Maximal stimulation occurs at a stoichiometric ratio of 1 monomer of gene 32 protein to 8–10 ssDNA nucleotides; at concentrations higher than this optimum, gene 32 protein strongly inhibits the reaction (Yonesaki and Minagawa, 1985). Since uvsY protein stabilizes the uvsX protein-ssDNA complex (Fig. 2), we wondered whether uvsY protein would also stabilize the complex in the presence of inhibitory amounts of gene 32 protein to promote the function of uvsX protein. This conjecture was prompted by the consideration that since synthesis of gene 32 protein is regulated by free ssDNA (von Hippel et al., 1983), in vivo recombination must proceed where intra-cellular ssDNA is, or is close to being, saturated by gene 32 protein.

Fig. 1. Binding of uvsY protein to ssDNA and dsDNA. A reaction mixture (25 μl) contained 20 mM Tris-HCl (pH 7.4), 7 mM MgCl2, 32P-labeled DNA, and uvsY protein as indicated in the abscissa. After incubation for 10 min at 37°C, the fraction of the protein-bound DNA was measured by DNA-cellulose column chromatography (Griffith and Formosa, 1985; Yonesaki and Minagawa, 1985). The results of this experiment shown in the figure indicate that (i) binding increases in a sigmoidal fashion as a function of uvsY protein concentrations, suggesting that the binding is cooperative, (ii) the protein binds better to ssDNA than to dsDNA, particularly at low concentrations of protein and DNA, and (iii) the protein binds stoichiometrically to ssDNA but not to dsDNA. The binding to dsDNA increases as a function of concentration of both protein and DNA. In contrast, binding to ssDNA is proportional to protein concentration, but independent of DNA concentration; binding reaches saturation when the ratio of ssDNA nucleotides to uvsY protein monomer approaches 14. We also examined binding of the protein to fd RFII (RFI linearized by a single cut with a restriction enzyme HpaI) and found no difference from RFI binding (data not shown). Further differences in the binding of uvsY protein to ssDNA and dsDNA are disclosed by the filter assay. First, 0.1 M NaCl does not affect the binding to ssDNA, but completely inhibits the binding to dsDNA. Second, the binding to ssDNA occurs rapidly and efficiently even at 0°C, whereas the binding to dsDNA is slow at 0°C (only 20–50% of the maximum binding is attained after more than 5 min) but rapid at 37°C (complete binding within 2 min). Finally, Mg2+ (7 mM) does not affect binding of the protein to ssDNA, but reduces that to dsDNA. From these observations, we conclude that uvsY protein has higher binding affinity for ssDNA than for dsDNA.

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uvX protein hydrolyzes ATP in the presence of ssDNA as a cofactor (Yonesaki and Minagawa, 1985; Formosa and Alberts, 1986). If the above surmise is the case, the ATPase activity will be stimulated by uvY protein and inhibited by an excess of gene 32 protein; furthermore, the inhibition induced by gene 32 protein should be relieved by uvY protein. As shown in Fig. 3, uvX protein continuously hydrolyzes ATP in the presence of fd DNA, whereas neither the uvX protein, the gene 32 protein, nor a mixture of uvX and gene 32 proteins has ATPase activity. The addition of uvX protein (1 monomer per 5 ssDNA nucleotides) to the reaction mixture containing uvX protein and ssDNA raises the catalytic activity of uvX protein 1.4-fold, and the addition of gene 32 protein (1 monomer per 0.4 ssDNA nucleotides) severely reduces the hydrolysis, as expected. The addition of gene 32 protein 4 min after the onset of the reaction leads to an immediate block in the reaction; residual activity is as low as that observed when gene 32 protein is added before the start of the reaction. The inhibition by gene 32 protein is completely relieved by the addition of uvY protein before or after the onset of the reaction apparently without lag. Fig. 3 also shows that until the substrate ATP is almost exhausted, the hydrolysis proceeds at a constant rate, even when the rate is increased by the addition of gene 32 protein and uvY protein. This implies that the reaction products very weakly interfere with the hydrolysis.

Renaturation of Complementary Single Strands in the Presence of Three Recombination Proteins—uvX protein renews complementary single strands using ATP as a cofactor (Yonesaki and Minagawa, 1985). Gene 32 protein is the first protein which was found to catalyze strand renaturation (Alberts and Frey, 1970). We had shown previously that renaturation mediated by uvX protein is much more efficient than that by gene 32 protein (Yonesaki and Minagawa, 1985). Renaturation products catalyzed by these proteins migrate at distinctly different velocities in agarose gel electrophoresis under our conditions (Fig. 4). When the product of uvX protein-mediated renaturation of heat-denatured fd RFIII was electrophoresed through 1% agarose, it did not enter the agarose gel (Fig. 4, lanes 3 and 4), probably forming network aggregates of strands (Yonesaki and Minagawa, 1985), while the product catalyzed by gene 32 protein ran at the same velocity as native RFIII and never formed aggregates (Fig. 4, lane 9), indicating that gene 32 protein catalyzes one-to-one pairing of complementary strands.

When we incubated heat-denatured RFIII with various amounts of uvX protein, in the presence of oversaturating amounts of gene 32 protein (1 monomer per 4.6 ssDNA nucleotides) and ATP, the products were found at the position of RFIII (lanes 10–12) even when the molar ratio of uvX protein to gene 32 protein was 1.2. In other words, the formation of “network” aggregates was suppressed by gene 32 protein. Thus, it is suggested that gene 32 protein binds to ssDNA preferentially or more strongly than uvX protein. uvX protein only very weakly promotes network DNA formation at low concentrations and not at all at high concentrations (data not shown). Lane 5 in Fig. 4 shows weak network formation at an optimal uvX concentration (1 monomer of uvX protein per 28.5 ssDNA nucleotides). At this concentration, uvX protein not only stimulates network formation by low concentration of uvX protein (lanes 6–8), but also remarkably eliminates the inhibition by gene 32 protein (lanes 14–16, cf. lanes 10–12).

The results described above strongly suggest that the small amount of uvX protein facilitates uvX protein binding to ssDNA even in the presence of an amount of gene 32 protein that otherwise would be sufficient to completely dissociate uvX protein from the complex.

Single Strand Assimilation in the Presence of Three Recombination Proteins—Single strand assimilation is one of the first steps in recombination and consists of strand separation of duplex DNA and pairing one of the strands of the duplex...
Fig. 4. Renaturation of heat-denatured DNA. A reaction mixture (12.5 μl) contained 20 mM Tris-HCl (pH 7.4), 12 mM MgCl₂, 1 mM ATP, and 11.4 μM heat-denatured fd RFIll (RFI linearized by a single cut with restriction enzyme HpaI) and proteins, of which concentrations are indicated below the figure. After a 20-min incubation at 30 °C, the reaction was terminated by the addition of 1.5 μl of a mixture composed of 2% SDS and 0.2 M EDTA, and kept further for 10 min. Then, 3 μl of a mixture of 40% glycerol and 0.02% bromphenol blue were added, and the sample was loaded onto a well of 1% agarose gel. Electrophoresis was performed in a buffer consisting of 18 mM Tris, 15 mM K₂HPO₄, and 0.5 mM EDTA for 2 h at 4 V/cm, and DNA bands were stained with 0.5 μg/ml ethidium bromide.

The natured DNA.

Fig. 5. Effect of uvsY protein on displacement of uvsX protein by gene 32 protein. ssDNA was first incubated with uvsX protein at 37 °C under four different conditions, in the presence or absence of uvsY protein (1 monomer per 10 ssDNA nucleotides) and of ATP (2 mM). One min after the beginning of the incubation, we added a large amount of gene 32 protein (1 monomer per 0.7 nucleotide) to all tubes. We then examined the strand assimilation activity of the aliquots at the times indicated in the abscissa in Fig. 5, by the addition of ³²P-labeled RFI and ATP, and further incubation for 12 min before the nitrocellulose membrane filtration assay. Fig. 5 shows that when gene 32 protein was added at the same time at which strand assimilation was initiated, all aliquots had the same high activity and there was no inhibition by gene 32 protein. When uvsY protein was included from the beginning, the activity in strand assimilation was maintained at a high level even after the addition of gene 32 protein, as expected. However, when ATP was present with uvsY protein, the activity was partially reduced. This reduction is based on extensive breakdown of ATP (cf. Fig. 3) before strand assimilation starts, which decreased ATP content available for the assimilation. When gene 32 protein was added in the absence of uvsY protein and ATP, there was a sudden and extensive reduction of the activity. Gene 32 protein inhibition was partially alleviated by ATP, consistently with our previous observations (Yonesaki and Minagawa, 1985). Activity was fully restored immediately after uvsY protein was added.

Single strand assimilation catalyzed by recA protein is strongly inhibited by ADP (Shibata et al., 1982). We found, however, that ADP or AMP does not inhibit the assimilation by uvsX protein even at concentrations up to 2-fold of that of ATP so far examined, consistent with the foregoing observation that products of ATP hydrolysis interfere very weakly with the ATPase activity of uvsX protein.

Efficient Single Strand Assimilation—We have shown that uvsY protein circumvents the inhibition of uvsX protein function by high concentrations of gene 32 protein. We show
now that the highest efficiency of single strand assimilation is attained by the simultaneous presence of all three proteins (Fig. 6). In the absence of uvsY and gene 32 proteins, strand assimilation is maximal at 1.3 mM uvsX protein. uvsY protein slightly increased this activity; the maximal activity is attained at a concentration of between 1.0 and 1.3 mM uvsX protein. In the presence of the optimal amount of gene 32 protein, the maximal activity of uvsX protein is at 0.65 mM. Finally, in the presence of uvsY protein and excess gene 32 protein, it is attained at as low as 0.33 mM uvsX protein. This same concentration of uvsX protein is ineffective when uvsY protein is omitted.

**DISCUSSION**

uvsX protein binds to ssDNA and dsDNA; the complex of uvsX protein with ssDNA plays a pivotal role in the formation of the in vitro synaptic intermediate in general recombination (Yonesaki and Minagawa, 1985; Formosa and Alberts, 1986; Harris and Griffith, 1987). uvsX protein is analogous to RecA protein of *E. coli*. Both gene 32 and uvsY proteins bind very strongly to ssDNA (Munn and Barry, cited by Alberts, 1984). Gene 32 protein, a helix destabilizing protein, essential for genetic recombination, indirectly promotes strand exchange (Yonesaki and Minagawa, 1985; Formosa and Alberts, 1986) and thus seems to be analogous to SSB protein of *E. coli*, although we do not exclude a direct involvement of gene 32 protein in recombination, in special cases such as in uvsW mutant-infected cells, in which abnormal amounts of gene 32 protein accumulate (Yonesaki and Minagawa, 1987). We have known neither the details of uvsY protein function nor an analogous protein in *E. coli*. In the present experiments, we show that this protein indirectly participates in strand exchange by stimulating the uvsX protein function.

In Fig. 7, we show schematically binding of these three proteins to ssDNA. uvsX protein cooperatively binds to ssDNA and assembles into filamentous forms (Griffith and Formosa, 1985; Yonesaki and Minagawa, 1985), and the binding is stable at 0 °C but unstable at 37 °C (Fig. 2), recA protein cooperatively binds to ssDNA unidirectionally from 5' to 3' along the DNA (Register and Griffith, 1985) and catalyzes strand displacement from 5' to 3' with respect to invading ssDNA (Cox and Lehman, 1981; West et al., 1981; Kaha et al., 1981). Since strand displacement by uvsX protein proceeds also from 5' to 3' along the DNA (Yonesaki and Minagawa, 1985), uvsX protein may polymerize with the same polarity by analogy with RecA protein. When uvsX protein separates from protein-ssDNA filaments, it may dissociate randomly along the filaments or in a polar manner from 5' to 3' along the ssDNA (Aa).

We do not know at present whether uvsY protein binds to ssDNA cooperatively or the morphology of the uvsY protein-ssDNA complex; we do know that uvsY protein exerts its biological functions at a low concentration and that it forms a complex with ssDNA that is stable at the physiological temperature (Ab). Although we have not determined exactly the minimum concentration of uvsY protein which exhibits biological function, 1 uvsY protein monomer per 57 ssDNA nucleotides stabilizes uvsX protein-ssDNA complex (Fig. 2), and 1 monomer per 28.5 ssDNA nucleotides promotes renaturation of heat-denatured RFII by uvsX protein in the presence of excess gene 32 protein (Fig. 4). This ratio is low when compared with that of the uvsX protein monomer which binds to 4.5 nucleotides (Griffith and Formosa, 1985) and with that of gene 32 protein which binds to 8–10 nucleotides (Newport et al., 1981). If uvsX protein binds cooperatively from the site contiguous to uvsY protein on a stretch of ssDNA, uvsX protein could block dissociation of uvsX protein from the DNA, resulting in the formation of longer lived uvsX protein-ssDNA complex without affecting the intrinsic affinity of uvsX protein for its lattice (Ac).

uvsX protein bound to ssDNA is efficiently displaced by gene 32 protein (Ba) and simultaneously loses its functional activity. This is shown, although indirectly, by experiments in Figs. 3–5. These results also indicate that, with the assistance of uvsY protein, uvsX protein is capable of binding efficiently to gene 32 protein-coated ssDNA. We imagine two pathways to produce such a complex by one (Bc) or two steps (Bb). We do not know at present which is the case or if both are correct. Recently, we have obtained evidence that uvsY protein is able to bind to gene 32 protein-coated ssDNA. The gene 32 protein-ssDNA complex migrates in a discrete band more slowly than free ssDNA in agarose gel by electrophoresis. After a brief incubation of the gene 32 protein-DNA complex with uvsY protein at 37 °C, the mobility of the band

**FIG. 6. Efficiency of uvsX protein in single strand assimilation.** A reaction mixture (20 µl) contained 20 mM Tris-HCl (pH 7.4), 12 mM MgCl₂, 2 mM ATP; 7.71 µM fd phage DNA fragment, 14.5 µM ³²P-labeled fd RFI (7.0 cpm/µmol), uvsX protein as indicated in the abscissa, or without uvsY protein and gene 32 protein. After a 15-min incubation at 37 °C, ³²P radioactivity retained on a membrane filter was counted. O, no uvsY protein and no gene 32 protein; •, 0.25 µM uvsY protein; □, 1.0 µM gene 32 protein; Δ, 3 µM gene 32 protein; ▲, 0.25 µM uvsY protein and 3.0 µM gene 32 protein.

**FIG. 7. Binding complexes of uvsX, uvsY, and gene 32 proteins with ssDNA.** A, effect of temperature on binding of proteins to ssDNA. B, dynamics of binding of proteins to ssDNA. •, uvsX protein; ▲, uvsY protein; O, gene 32 protein; and —, ssDNA. See details in the text.
is altered by as little as 1 monomer of uvsY protein per 45 ssDNA nucleotides. It is not known whether binding of uvsY protein to gene 32 protein-coated ssDNA is accomplished by addition, insertion, or displacement; however, uvsX protein becomes functional after uvsY protein-mediated binding to ssDNA. According to experiments of ATPase (Fig. 3) and strand assimilation (Fig. 5), displacement of uvsX protein by gene 32 protein and uvsY protein-mediated binding of uvsX protein to gene 32 protein-coated ssDNA are very rapid actions that occur without delay after the addition of the protein(s).

uvsY protein confers stability to uvsX protein-ssDNA complex under conditions such as at physiological temperature and in the presence of excess gene 32 protein, enhancing ssDNA-dependent hydrolysis of ATP, renaturation of heat-denatured DNA, and single strand assimilation into homologous dsDNA. These functional characteristics account for the similarity in phenotypes of mutants in uvsX and uvsY genes and are very important for the function of uvsX protein in T4-infected cells, in which ssDNA is coated with gene 32 protein and uvsY genes and are very important for the function of uvsX protein in T4-infected cells, in which ssDNA is coated with gene 32 protein (Curtis and Alberts, 1976). We have recently found that plasmid-born uvsX gene slightly increases UV resistance of recA gene mutants of E. coli, and genetic recombination of λ phage and E. coli, and weakly interferes with the induction of the SOS response of E. coli and of λ prophage. Both the increase and the interference are dramatically enhanced by the co-existence of a cloned uvsY gene in the cell. The cloned uvsY gene alone shows no detectable activity (Minagawa et al., 1988). Therefore, the present results on the in vitro function of uvsY protein are in good agreement with those observations on in vivo functions of uvsY gene.

How does gene 32 protein assist the uvsX protein function? We examined the effect of concentrations of gene 32 protein upon uvsX protein-mediated strand assimilation in the presence of uvsY protein. Increasing the ratio of gene 32 protein monomers to ssDNA nucleotide from 0 to 2 enhanced the reaction, the maximum being at a ratio of 2. Since one gene 32 protein monomer binds to 8–10 ssDNA nucleotides (Newport et al., 1981), in the presence of uvsY protein, the maximum of uvsX protein function is reached at large excess of gene 32 protein. The presence of excess gene 32 protein may favor dispersive binding of uvsY protein to ssDNA and may reduce the size of uvsX protein-ssDNA filaments, which leads to the formation of many scattered short uvsX protein filaments. The other explanation, although not mutually exclusive, is that unwinding of dsDNA and unfolding of secondary structure in ssDNA, which are a prerequisite for strand displacement, are carried out by gene 32 protein. The more gene 32 protein is included, the more efficiently base pairs may be eliminated. For instance, hairpin structures in ssDNA are removed by cooperative binding of gene 32 protein. If the strand is coated with gene 32 protein, uvsY protein can bind to the strand and promote successive polymerization of uvsX protein at the contiguous site on the DNA. Extended uvsX protein-ssDNA filaments stabilized by uvsY protein at one end and ready to be assimilated are generated. This explanation can be derived from the above observation and the observation that uvsX protein does not catalyze single strand displacement unless gene 32 protein is supplied, although recA protein does without any other protein (Yonesaki and Minagawa, 1985; Formosa and Alberts, 1986). Differing from recA protein, uvsX protein unwinds dsDNA very weakly, if at all, in the presence of ATP. When unwinding activity is examined by using covalently closed relaxed DNA of fd-plage as a substrate and calf thymus topoisomerase I (Bethesda Research Laboratories) to trap superhelicity, uvsX protein maximally causes reduction of linking number only by one to two in the presence of ATP regardless of whether homologous circular ssDNA is included or not. The above model would be able to explain why T4 recombinase is promoted by uvsX gene but not by host recA gene, even when the latter gene product is overproduced (Yonesaki et al., 1984; Conkling and Drake, 1984). This is neither because recA protein is degraded in a cell after infection, nor because purified recA protein cannot catalyze reactions involving DNA in which cytosine residue is modified as is in T4 DNA (Yonesaki and Minagawa, 1988). recA protein in T4-infected cell extract does not bind to the gene 32 protein column (data of Formosa et al., 1983), suggesting that recA protein does not bind to gene 32 protein by itself or with the help of other proteins which have affinity to both proteins. It could be that after T4 infection gene 32 protein binds to ssDNA and impedes binding of recA protein to ssDNA. This is also supported by the recent finding that, in the absence of gene 32 protein, the cumulative effect of recA, uvsX, and uvsY genes is evident on genetic recombination of λ phage and E. coli in a cell, which bears cloned T4 genes (Minagawa et al., 1988).

From the individual functions and properties of uvsX, uvsY, and gene 32 proteins, we believe that they constitute the simplest form of the recombination machine of bacteriophage T4. Recently, Kodadek and Alberts (1987) have reported that the dda gene product powerfully promotes strand displacement in the presence of uvsX and gene 32 proteins. The reaction is dependent on uvsX protein, but the requirement for gene 32 protein is not mentioned. These results strongly indicate that destabilization or unwinding of dsDNA by dda protein or gene 32 protein, or the coupled action of these proteins, augments strand displacement. dda protein has a helicase activity and can unwind a long DNA molecule (Krell et al., 1979). Furthermore, it binds strongly to the gene 32 protein affinity column, more strongly than gene 32 protein itself or uvsY protein (Formosa et al., 1983). These characteristics of the protein suggest that the protein is also a component of T4 recombination machinery (Kodadek and Alberts, 1987). According to Behme and Ebisuzaki (1975), dda gene is dispensable for phage growth, and its mutation does not affect genetic recombination and UV sensitivity in ordinary strains of E. coli. Further investigation seem to be required to decide whether dda protein is an essential component of the in vivo machinery or not. So far, the observations suggest that recombination in T4 is carried out by a class of proteins with a specific function and structure as has been found for other processes involving DNA.

recA protein is multifunctional and participates in various biological phenomena. In contrast to uvsX protein, recA protein extensively unwinds dsDNA in the presence of ATP as well as ATPγS (Wu et al., 1983). recA protein has a proteolytic activity, but uvsX protein does not (Yonesaki and Minagawa, 1985; Minagawa et al., 1988). The interaction of recA protein with ssDNA and SSB protein appears to be more complex than that of uvsX protein with ssDNA and gene 32 protein. In the meantime, recA-like protein or activity has been found also in many eukaryotic organisms, for instance, Ustilago (Kmiec and Holloman, 1986), yeast (Colodner et al., 1987; Sugino et al., 1988), mouse (Hotta et al., 1985), human (Hsieh 1987).
et al., 1986; Cassuto et al., 1987), and lily (Hotta et al., 1985).
As is well known in eukaryotes, DNA is bound to proteins more tightly and in a more complicated manner than in prokaryotes. This fact and present results may suggest that uvsY protein-like accessory protein is needed for the eukaryotic enzymes to function. Recombination proteins of T4 phage, because of simpler properties, will provide us with a better tool to investigate the mechanism and machinery of strand exchange.

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