Process of Infection with ϕX174: Effect of Exonucleases on the Replicative Form

Abstract. *When a preparation containing the DNA of the bacteriophage ϕX174 in its replicative form together with DNA from* Escherichia coli *is treated with exonucleases, the replicative form retains its characteristic physical properties, while the bacterial DNA is degraded. The evidence suggests that the mature replicative form exists as a closed ring.*

When cultures of *Escherichia coli,* strain C, are infected with bacteriophage ϕX174 in the presence of chloramphenicol (25 μg/ml), the single-stranded DNA of the bacteriophage (*1*) is converted to a replicative form (RF) (*2*). The RF differs from the single-stranded DNA with respect to buoyant density, melting behavior, and resistance of its infectivity—in the protoplast assay (*3*)—to ultraviolet irradiation (*2, 4*). In the presence of chloramphenicol, the amount of this form increases with time, without appreciable synthesis of single-stranded DNA and with no production of progeny virus particles (*2*).

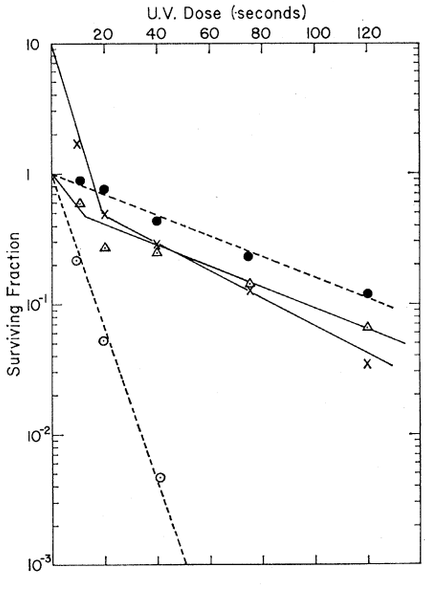

Fig. 1. Inactivation by ultraviolet irradiation of the infectivity of DNA preparations. Residual infectivity is plotted as a function of the ultraviolet dose (from a germicidal lamp at 30 cm). Solid circles, purified RF preparation; dotted circles, standard single-strand DNA preparation; dotted triangles, DNA preparation from infected cells before exonuclease action; crosses, DNA preparation from infected cells after successive action of exonucleases III and I. Infectivity plotted relative to infectivity before action of enzymes.

During purification of the RF from a phenol extract of such infected cells, a nucleic acid fraction is obtained which is composed principally of *E. coli* DNA and which contains approximately 25 percent of the infectivity of the extract. Most of the material infective to protoplasts (*3*) shows the ultraviolet sensitivity (Fig. 1) and buoyant density (Fig. 2) characteristic of the RF. When this preparation is treated successively with *E. coli* exonuclease III (*5*) and *E. coli* exonuclease I (*6*), nearly 70 percent of the host DNA is degraded to acid-soluble fragments,

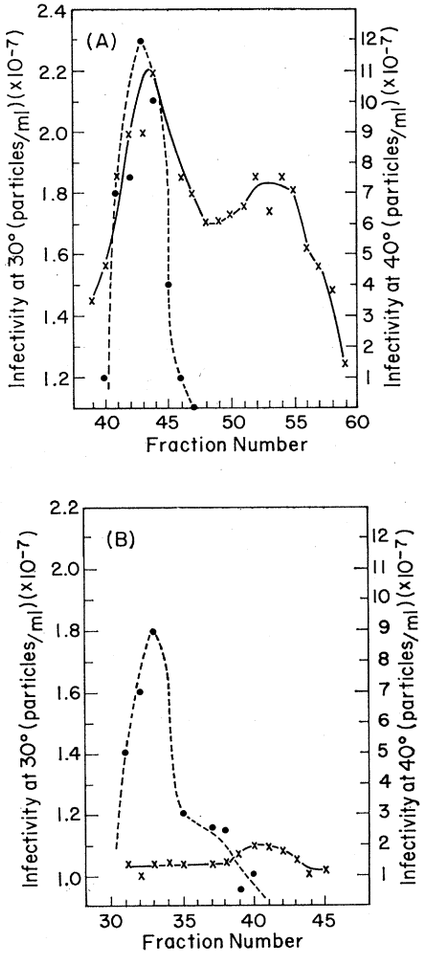

Fig. 2. Density distribution of infectivity in a CsCl gradient. Infectivity is expressed in terms of the equivalent concentration per milliliter of wild-type single-strand ϕX174 DNA molecules. As a density marker, single-stranded DNA from a double mutant, γh_1, of ϕX174 is used. γh_1 DNA has the same buoyant density as wild-type ϕX174 DNA; both species may be assayed independently (wild type on *E. coli* C at 40°C, γh_1 on *E. coli* C, at 30°C). (*A*) Crosses, infectivity of exonuclease-treated DNA preparation; solid circles, infectivity of γh_1 marker DNA. (*B*) Crosses, infectivity of DNA preparation before enzyme treatment; solid circles, infectivity of γh_1 marker DNA.

while the remainder consists of small pieces which are not retained on a methylated-albumin chromatographic column (*7*). The infectivity of the preparation, however, increases 10 to 20 fold (Fig. 3). The substance associated with the infectivity has largely the ultraviolet sensitivity and buoyant density characteristic of single-stranded DNA (Figs. 1 and 2). A smaller increase in the amount of infectivity with the characteristics of the RF is also observed (Fig. 2).

Since there can be severe inhibition of the biological assay of the RF by *E. coli* DNA, all assays of infectivity are performed at sufficient dilution that infectivity is directly proportional to concentration. For this reason, it is not likely that the observed increase in infectivity is due simply to the removal of the *E. coli* DNA. Infective material is apparently released as a consequence of enzymatic action either on viral DNA forms of considerably lower infectivity than the single-stranded DNA or on complexes of viral and *E. coli* DNA.

The persistence of the infectivity of released single-stranded DNA during

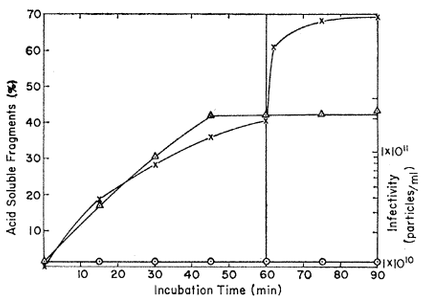

Fig. 3. Effect of exonuclease action upon the solubility in acid and the infectivity of the DNA fraction from infected cells. Acid solubility is measured as the percentage of the initial absorbancy. $A_{260}m\mu$ ($\times$ 1.4 to correct for hyperchromic effects), of the preparation which remains soluble in 6 percent perchloric acid in the presence of 150 μg/ml of thymus DNA carrier. For enzyme digestions, DNA (230 μg/ml) is treated with exonuclease III (220 units/ml) in 0.067*M* tris buffer, *p*H 8.0 plus 0.00067*M* $MgCl_2$ plus $10^{-3}M$ mercaptoethanol. Enzyme action is halted in samples, removed at various times, either by dilution and chilling (for infectivity assay) or by addition of acid (for absorbance measurement). After 60 minutes, the digest is chilled and dialyzed against 0.066*M* glycine buffer, *p*H 9.5, containing 0.014*M* NaCl and $10^{-3}M$ $MgCl_2$. For further digestion, 40 units of exonuclease I per milliliter are added. Crosses, percentage acid-soluble nucleotides; triangles, infectivity of enzyme-treated DNA fraction; circles, infectivity of control (enzymes omitted).

the extensive degradation of host DNA by these enzymes is consistent with the previous evidence that the ϕX174 DNA is a ring structure, resistent to exonuclease attack (*8*). That the RF also resists degradation under these conditions suggests that it, too, is a ring. In confirmation of these results, recent electron micrographs (*9*) of RF preparations show the presence of ring structures of the expected contour length (*10*).

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Complementary Strand Association between Nucleic Acids and Nucleic Acid Gels

Abstract. *Nucleic acid gels can be formed as a result of the cross-linking action of ultraviolet light or of nitrous acid. Such gels form duplex combinations with complementary nucleic acid strands.*

Deoxyribonucleic acid, immobilized on cellulose or in agar, has been used for the isolation of complementary ribonucleic acid (*1, 2*) and also in studies of DNA-DNA interactions (*3, 4*). That nucleic acids (*5–7*) themselves can be made into insoluble gels by ultraviolet-induced cross linkages suggests another general method for the preparation of materials suitable for complementary strand association studies. This report describes further observations on the formation of such gels by the actions of ultraviolet light or nitrous acid as well as tests of the resulting gels as practical experimental tools. It appears that any naturally occurring nucleic acid and most artificial nucleotide polymers, including oligonucleotides (*7*), can be readily converted to such gels.

A simple qualitative procedure has been used to test the formation of gels with a variety of nucleic acids. A drop of water containing about 50 μg of nucleic acid was dried at 100°C on a microscope slide. While drying the solution was distributed with a glass rod over an area of about 2 cm^2. The cover slip was irradiated for several minutes 5 cm from a General Electric 15-watt germicidal lamp. The slide was rinsed and scraped lightly in 3 ml of water to remove the gel and to dissolve any ungelled polymer. When DNA, ribosomal RNA, soluble RNA, or polyU (*8*) were treated in this way, visible fibers were formed. Upon irradiation for only a few seconds the gel structure was so open that it was barely visible in water, but the addition of 0.01*M* $MgCl_2$ condensed it into visible fibers. At higher doses the fibers appeared to be very dense even when suspended in distilled water. An estimate of the quantity of ungelled polymer could be made by filtering the gel and measuring the optical density of the filtrate at 260 mμ. The amount of irradiation required to form a gel depends on the molecular weight of the polymer, the optical thickness of the dried material, and the fraction of bases present capable of forming dimers.

Gels may also be formed on inert supporting materials such as polyvinyl beads (*9*), cellulose fibers and nylon thread. Figure 1 shows the degree of retention of polyU and polyC on Geon 101 resin grains after drying the polymers out of water solution and exposing them to ultra-violet light 22 cm from the 15-watt germicidal lamp. The curves are based on the reduction in optical density of the filtrate after the resin was suspended in water and washed thoroughly on a glass-wool filter. Other measurements have shown that for this dose range there is no significant reduction of the optical density of the total polyU or polyC resulting from dimer formation or other chemical effects of ultraviolet irradiation. At considerably higher doses such effects are significant. There was no evidence for gel-formation of polyA or polyI at these doses of irradiation. At a dose of 10^7 ergs/cm^2 there was a 30 percent reduction in the optical density of the polyA filtrate, which may have been due to chemical effects reducing the total optical density of the polyA.

The DNA gels could also be formed by treating dried films (previously made single-stranded by boiling in water solution) with nitrous acid (*10*). For this purpose 0.25*M* KNO_2 was adjusted to *p*H 4.2 by the addition of acetic acid and it was diluted with 2 volumes of ethanol. A few drops of this solution were placed on the dried film and allowed to remain for 10 minutes. The gel was then washed off in tris buffer, *p*H 7.4. Fibers were observed with properties comparable to those formed upon ultraviolet irradiation.

It was possible to obtain specific complementary strand association between RNA from bacteria and DNA gels formed by cross linking as a result of ultraviolet irradiation. In one experiment 3.5 mg of DNA was dissolved in water and boiled for 10 minutes to separate the strands. The solution was brought to 0.15*M* NaCl containing 0.015*M* sodium citrate (SSC) and 100 mg of cellulose powder added to supply bulk for convenient handling. Two volumes of alcohol were added and the wet precipitate was exposed to ultraviolet light (2 minutes with stirring 5 cm from the 15-watt germicidal lamp). The bulk of the DNA did not precipitate in sufficiently close association with the cellulose fibers, and as a result only about 10 percent of the original DNA remained in the cellulose column after thorough

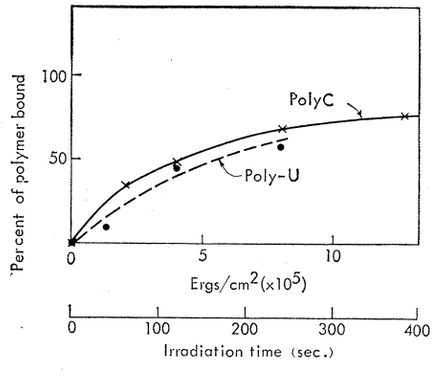

Fig. 1. Fraction of polyU and polyC immobilized on plastic beads as a function of total ultraviolet dose. Eight milligrams of polymer were dried on 2 g of Geon 101 resin. Samples (100 mg) were given four exposures with mixing in between each. The fraction bound was determined from the reduction in optical density of all material that could be washed from the resin. The scale showing ergs/cm^2 is based on a National Bureau of Standards calibration of the energy emitted in the Hg line at 254.7 mμ from an unfiltered lamp. The 254.7 line presumably is the effective radiation causing cross linking by dimer formation.