T4 Infection and DNA Preparation

Bacterial cells were grown with vigorous shaking in L broth at 37°C to a cell density of 4x10^8 per mL (OD_{560} 0.5), and infected with the indicated phage strain at a multiplicity of 6 plaque-forming units per cell. After 4 minutes at 37°C without shaking (for adsorption), incubation was continued with shaking up to the indicated time. For each time point, infected cells from 1 mL of culture were collected by centrifugation at 13,000 rpm for 2 minutes and resuspended in 300 μL of lysis buffer (50 mM Tris-HCl [pH 7.8], 10 mM disodium EDTA, 100 mM NaCl, 0.2% SDS). Proteinase K was added to 0.5 mg/mL, and the resuspension was incubated at 65°C for 2 hours. Total nucleic acid was extracted sequentially with phenol and chloroform-isooamyl alcohol (24:1), then dialyzed overnight at 4°C against TE buffer (10 mM Tris-HCl [pH 7.8], 1 mM disodium EDTA).

2D Agarose Gel Electrophoresis

Total DNA from infected cells was treated with restriction endonuclease PacI (New England Biolabs). Samples were analyzed by 2D agarose gel electrophoresis according to Friedman and Brewer (1995) with minor modifications, as described previously (Long & Kreuzer, 2008). 2D gels were visualized by Southern blot with a probe specific to the ori(34) region (T4 coordinates 149172-152033).

DSE Accumulation Assay

Total DNA from infection was treated with restriction endonuclease AseI (New England Biolabs) prior to separation on a 1% agarose gel run in 0.5X TBE buffer for 12 hours at 2 V/cm at room temperature. The gel was visualized by Southern blot with a probe that is specific to a region downstream of ori(34) (T4 coordinates 151767-152688).
Southern Blots

Agarose gels were transferred to a 0.45 μm Nytran transfer membrane (Whatman, Inc.) by the downward sponge method (Ming et al, 1994). Following transfer, DNA was crosslinked to the membrane with a 120 mJ/cm² UV exposure. Radiolabeled probes were generated using the Random-Primed DNA Labeling kit (Roche) and the appropriate PCR fragment. All blots were visualized using the Storm 860 Phosphorimager (Molecular Dynamics) and quantitated with ImageQuant software (Molecular Dynamics).

Supplemental Figure Legends:

SI Figure 1. 2D gel analysis of chromosomal ori(34) during T4 infection. Total DNA from the indicated infections was digested with PacI and separated by 2D gel electrophoresis. Gels were visualized by Southern blot with a probe specific to the ori(34) region.

SI Figure 2. Partial resolution of total DNA by UvsW produces a cone-shaped region in 2D gels. Total DNA (from 49 phage, 9 minutes post infection) was subjected to increasing amounts of interstrand DNA crosslinking with trioxsalen (0, 50, 100, 200 nM) prior to incubation with UvsW at 37°C in 1X reaction buffer. Interstrand DNA crosslinks prevent branch migration, thereby trapping partially resolved intermediates for visualization by 2D gel. A schematic of the predicted migration of regressed fork structures (from along the entire Y-arc) is shown at right.

References for Supplemental Material:


