Supporting Information

Long and Kreuzer 10.1073/pnas.0711999105

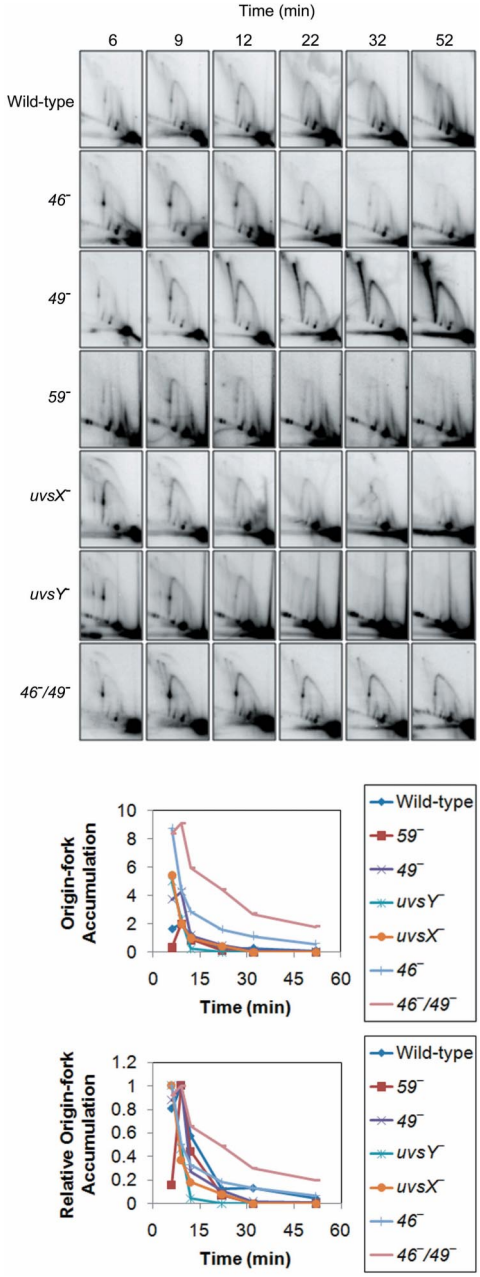

Fig. S1. Complete 2D gel time courses from T4 infections. The first-dimension gel consisted of 0.4% agarose (Shelton Scientific) run in 0.5× TBE buffer [44.5 mM Tris·HCl, 44.5 mM borate, and 1 mM disodium EDTA] for 30 h at 0.75 V/cm at room temperature (21°C). The desired lane was then sliced from the first-dimension gel and cast across the top of the second-dimension 1% agarose gel, which was run in 0.5× TBE buffer containing 0.3 μg/ml ethidium bromide for 16 h at 4.5 V/cm at 4°C. The resulting 2D gel was visualized by Southern blot with a probe specific to the *ori(34)* region (T4 coordinates 149172–152033). In addition to the branched DNA forms described in the text, two additional arcs are visible in some of these panels: (*i*) the theta arc of bubble intermediates, which migrates above the Y arc in the second dimension [Doan PL, Belanger KG, Kreuzer KN (2001) Two types of recombination hotspots in bacteriophage T4: One requires DNA damage and a replication origin and the other does not. *Genetics* 157:1077–1087] and (*ii*) a faint arc to the left of the origin fork/comet in the first panel of the *uvsX* and *uvsY* mutant infections. We believe this arc arises from incomplete restriction digestion of the normal origin fork fragments. The large accumulation of X-shaped molecules (presumptive Holliday junctions) in the *49*$^-$ mutant infection is largely absent in a *49*$^-$ *uvsX*$^-$ double mutant (data not shown), indicating that these intermediates are formed predominantly in a UvsX-dependent reaction.

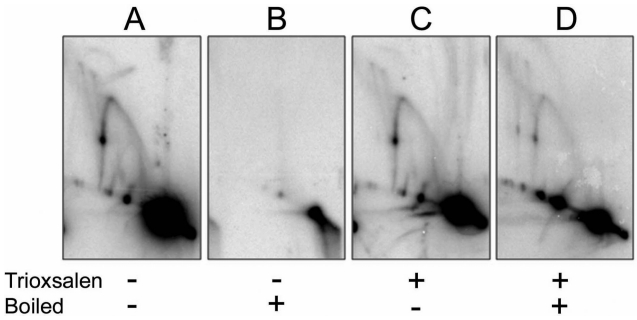

Fig. S2. Regressed forks from DNA samples cross-linked *in vivo*. (*A–D*) T4-infected cells were treated with 2.5 μM Trioxsalen (*C* and *D*) or a similar volume of methanol without Trioxsalen (*A* and *B*) on ice for 1 h to allow incorporation. Cells were exposed to long-wave UV light for 20 min and then collected by centrifugation and resuspended in 300 μl of lysis buffer. DNA preparation was performed as described in *Materials and Methods* and then analyzed by 2D gel with (*B* and *D*) or without (*A* and *C*) pretreatment at 100°C for 5 min.

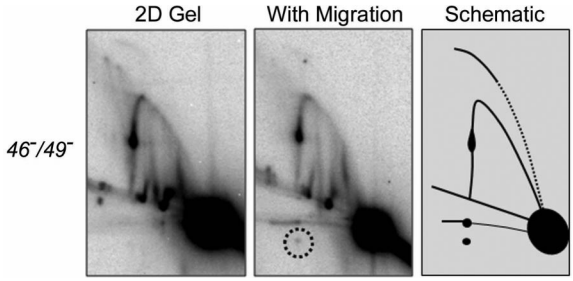

Fig. S3. Extrusion of regressed duplex DNA during 2D gel electrophoresis. For the gel labeled ''With Migration,'' the first-dimension gel slice was soaked in migration buffer at 65°C for 4 h to promote spontaneous branch migration within the gel. The assay was performed with PacI-digested DNA from *46*$^{-/49-}$ infection at 9 min. The 2D gels that favor extrusion of regressed forks were run essentially as described previously [Fierro-Fernandez M, Hernandez P, Krimer DB, Schvartzman JB (2007) Replication fork reversal occurs spontaneously after digestion but is constrained in supercoiled domains. *J Biol Chem* 282:18190–18196]. A first-dimension gel slice containing the DNA sample of interest was soaked in migration buffer [10 mM Tris·HCl (pH 8), 0.1 mM disodium EDTA, and 100 mM NaCl] at 65°C for 4 h. The second-dimension gel was then cast and run as described in *Materials and Methods* and the legend to Fig. S1. Extensive branch migration of a regressed fork in the time between the first- and second-dimension gel electrophoresis can result in complete extrusion of the regressed arm (dotted circle) and generation of a full-length linear restriction fragment. An interpretive diagram is shown to the right of the ''With Migration'' gel. Although there may be a small amount of spontaneous fork regression in the leftmost panel, quantitation shows ≈3-fold more intense signal in this area in the ''With Migration'' gel.