Supplementary Materials and methods

DNA templates

pORIR1 plasmid was obtained by cloning by cloning λ DNA sequence fragment (four iterons and AT-rich region of *ori*λ) into pUC19 vector. λ fragment was obtained by PCR using primers 5'-CCGAGCTCTAACACCGTGCGTGTTGAC and 5'-CCGGATCCAGAGGATTCGCCAGAATTC and cloned into SacI/BamHI sites of pUC19. Subsequently, *lac* promoter sequence was removed by digesting obtained plasmid with SapI and HindIII and replacing the 243bp fragment with a linker containing p_R promoter sequence (transcription starting from this promoter oriented away from the AT-rich segment). The linker was obtained by hybridization of oligomers (5'-GGAAGCGGAAGAGCTCCTTAGTACATGCAACCATTATCACCGCCAGAGGTAAAATAGTCA and its complementary counterpart).The resulting plasmid was sequenced and its supercoiled form was purified by centrifugation in CsCl-ethidium bromide equilibrium gradient.
DNA fragment used as a competitor DNA containing iteron sequences was obtained by PCR using primers: 5' – biotin – TCA AGC AGC AAG GCG GCA TGT TTG G-3' and 5' TGT CCC CCT GTT TTG AGG GAT AG – 3.

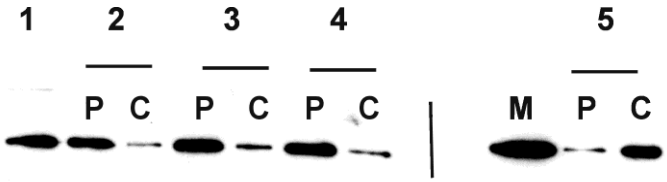
Gel shift experiments

Reactions (final volume 25 μl) were performed in a buffer containing : 10 mM Tris-HCl pH 7.6; 10 mM MgCl2 ; 20 mM KCl; 0.5 mM DTT; 0.1 mM EDTA and 50 μl/ml poly (dI-dC). 1 μg of a supercoiled plasmid DNA was mixed with *E. coli* RNA polymerase (250 ng) and incubated for 10 min at 30°C. Subsequently, 600 ng of λO protein was added and incubation was continued for another 10 min. After this period, NTPs were added (500 μM each), and incubation was carried on for another 10 min. 4 μl of loading buffer (30% v/v glycerol, 0.3% w/v bromophenol blue) was added to each sample and protein-DNA complexes were subsequently separated electrophoretically in a 0.8 % agarose gel in 0.5 x TBE buffer at 4°C. Bands were visualized by ethidium bromide staining and photographed under UV light. Alternatively, glutaraldehyde was added to the nucleoprotein complexes mixture (to a final concentration of 0.03 %) and incubation was prolonged for the next 10 min. Cross-linking products were resolved separated electrophoretically in a 7.5 % SDS-polyacrylamide gels, transferred onto PVDF membrane (Millipore) and detected by immunoblot analysis using anti-λO polyclonal antibodies and HRP-conjugated anti-rabbit secondary antibodies. An ECL-Western Blotting Substrate Kit (Pierce) was used to detect antibody-antigen complexes.

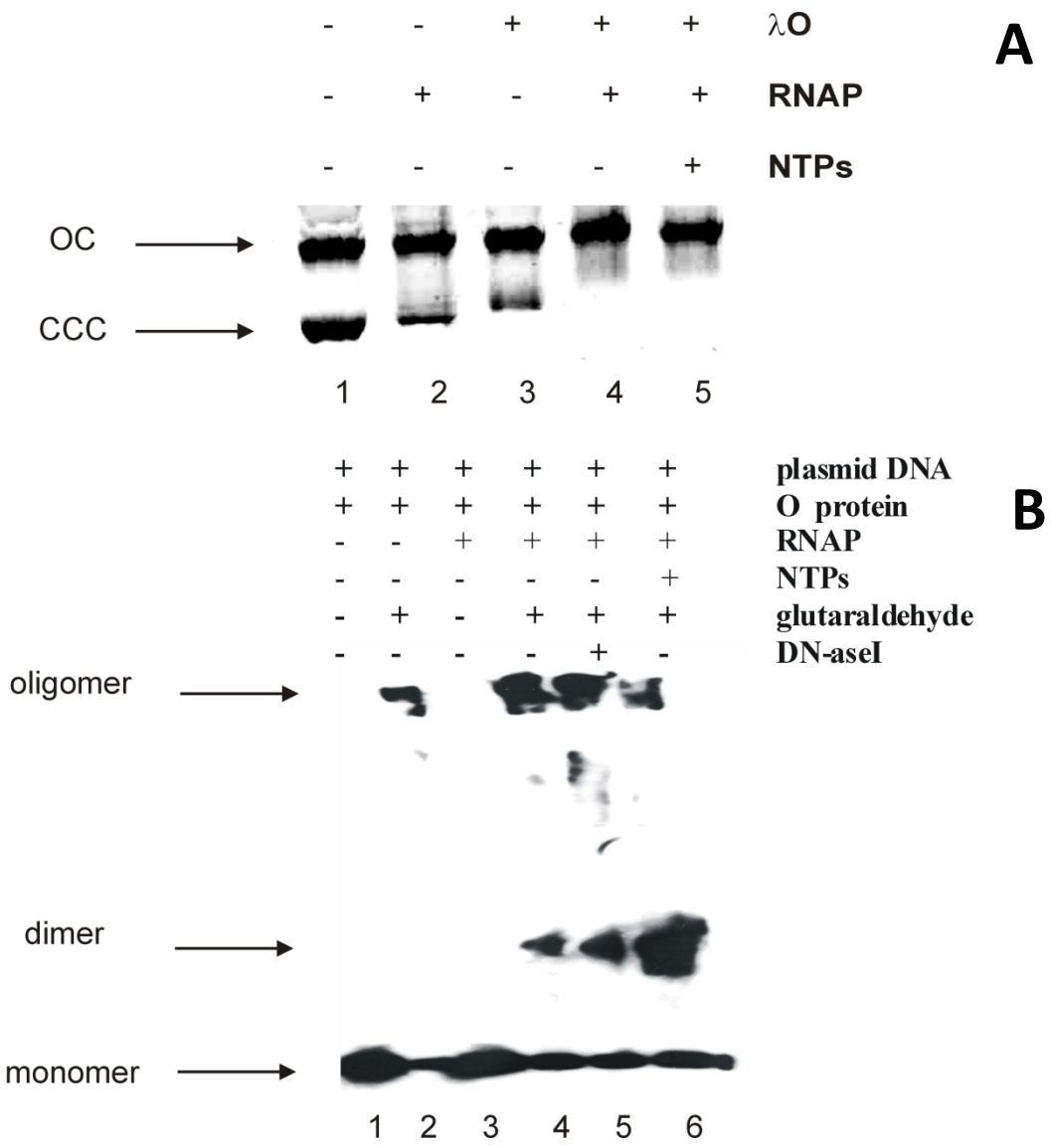
Stability of O-some complexes - competitor challenge

The experiment was performed using streptavidine-coated magnetic beads (Sigma). Magnetic beads (10 μl per further reaction) were washed three times in a reaction buffer (10 mM Hepes-KOH pH 7.6; 10 mM $MgCl_2$; 20 mM KCl; 0.5 mM DTT , 0.1 mM EDTA) and resuspended in this buffer in 1/2 of the initial volume. Subsequently, supercoiled pORIR1 plasmid DNA was incubated with λO protein in 1:10 molar ratio (1,25 pmol plasmid DNA and 12,5 pmol protein per further reaction) for 10 min at 30˚C. Unbound protein was removed by incubation with excess of biotinylated competitor DNA containing iteron sequences (molar ratio 5:1 competitor : plasmid DNA) and O-some complexes were separated from competitor DNA using magnetic beads (15 min incubation at 30˚C with mixing). O-some complexes were divided to four samples. To first , 4× SDS-PAGE loading buffer was added, third and fourth sample were supplemented with RNA polymerase diluted in 1× reaction buffer (60 nM final

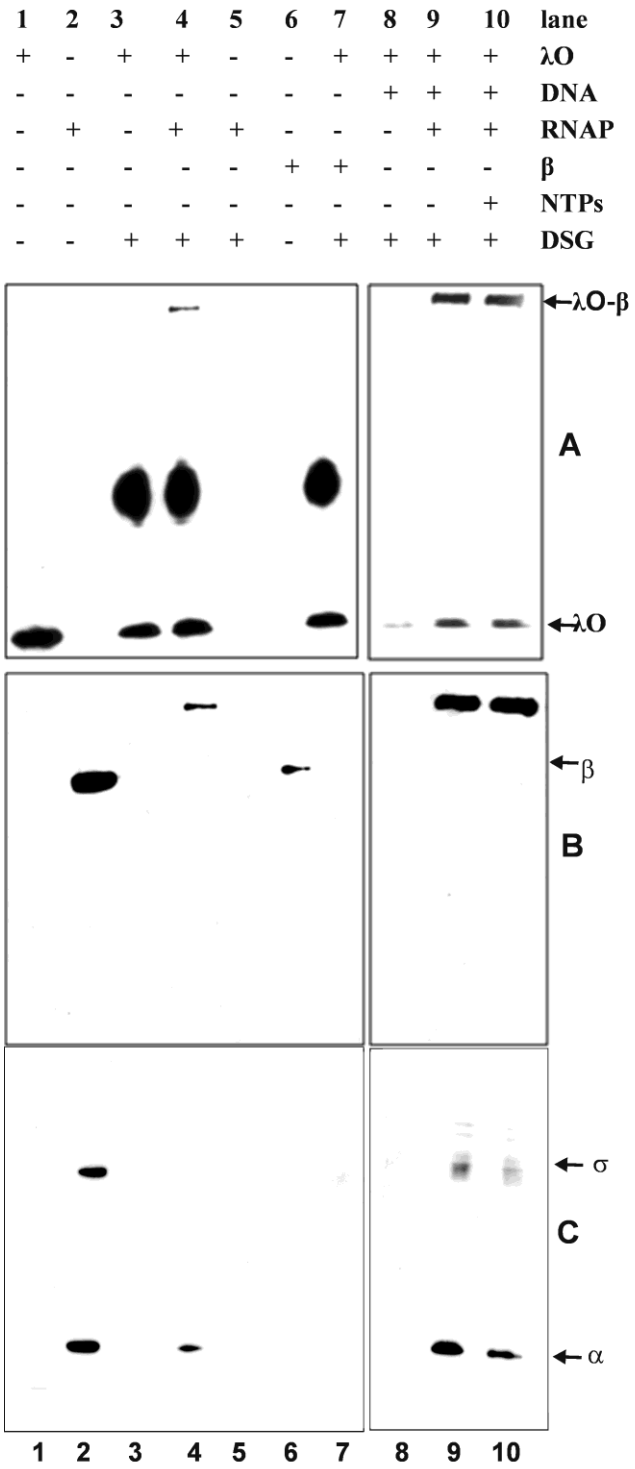
concentration) and to the second appropriate amount of 1×buffer was added and the three samples were further incubated for 10 min at 30˚C. After that time competitor DNA was added to the three samples in 50 × molar excess over plasmid DNA and to the fourth sample competitor DNA was added together with NTPs (0,5 mM each) and incubation was continued for 15 min. Final reaction volume was 25 µl. Reactions were subsequently supplemented with a suspension of magnetic beads in 1× reaction buffer. After binding of competitor DNA to beads (15 min at 30˚C with mixing), beads were separated on a magnetic separator, SDS-PAGE loading buffer was added to supernatant (containing plasmid-bound λO) and beads were washed with 30 µl of reaction buffer and resuspended in the same volume of the buffer. Separately, a sample was prepared (5) in which λO protein was incubated simultaneously with plasmid and competitor DNA for 15 min at 30˚C before addition of magnetic beads, and subsequently it was proceeded as described for other samples. All fractions were separated electrophoretically in a 7.5 % SDS-polyacrylamide gels, transferred onto PVDF membrane (Millipore) and detected by immunoblot analysis using anti-λO polyclonal antibodies and HRP-conjugated anti-rabbit secondary antibodies. An ECL-Western Blotting Substrate Kit (Pierce) was used to detect antibody-antigen complexes.



Supplementary Figure 1. Influence of RNA polymerase activity on stability of O-some structure in the presence of excess of competitor DNA was assessed. M- λO, P- plasmid-bound fraction of the λO protein remaining after competitor challenge; C- fraction of the λO protein which bound to the competitor DNA. 1- input of the plasmid-bound λO protein, 2- O-some +competitor, 3- O-some + RNAP + competitor, 4- O-some + RNAP + NTPs + competitor, 5- λO was incubated simultaneously with the plasmid and competitor DNA, to show effective competition by this DNA fragment. In this case P and C stand for λO protein that bound to the plamid and competitor DNA during incubation. The experiment was performed as described in Supplementary Materials and Methods.



Supplementary Figure 2. **A.** λO protein binding to λ plasmid DNA in the presence and absence of RNA polymerase and NTPs. Electrophoretic mobility shift assay was performed with supercoiled λ plasmid (pRLM4). λO protein was incubated with DNA for 10 min at 30°C, subsequently RNA polymerase and NTPs were added (where indicated) and incubation was continued for another 10 min at 30°.Samples were separated in a 0.8 % agarose gel in 0.5 x TBE buffer. Bands were visualized by ethidium bromide staining. B. nucleoprotein complexes were formed as described above but they were subsequently cross-linked with glutaraldehyde, separated by SDS-PAGE and detected by immunoblot analysis with anti-λO antibodies. Lane 5- sample was subjected to DN-aseI digestion prior to electrophoresis. The position of supercoiled plasmid DNA (CCC) and nicked circular DNA (OC) was marked.



Supplementary Figure 3. β subunit of RNA polymerase makes a direct contact with both iteron-bound λO and a free form of the λO protein.

λO (0,5 μM) was bound to *ori*λ-containing plasmid (2,8 nM) and separated from unbound protein fraction by gel filtration. Subsequently, reaction was supplemented with RNA polymerase (22 nM) and subjected to DSG cross-linking in the presence or absence of nucleotides (500 μM). Free λO protein was also subjected to DSG cross-linking with RNA polymerase. Subsequently, protein complexes were resolved by SDS-PAGE and detected by immunoblotting, performed with polyclonal antibodies specific against λO (A) or monoclonal antibodies against β subunit of RNAP (B) or simultaneously against α and σ subunits (C). The presence or absence of each reaction component is indicated. λO-RNAP polymerase complex was depicted by an arrow.