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# A Polycomb complex remains bound through DNA replication in the absence of other eukaryotic proteins

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**Propagation of chromatin states through DNA replication is central to epigenetic regulation and can involve recruitment of chromatin proteins to replicating chromatin through interactions with replication fork components. Here we show using a fully reconstituted T7 bacteriophage system that eukaryotic proteins are not required to tether the Polycomb complex PRC1 to templates during DNA replication. Instead, DNA binding by PRC1 can withstand passage of a simple replication fork.**

Epigenetic regulation through chromatin structure requires chromatin based information to be propagated through cell cycles. During DNA replication, chromatin structure, including chromatin bound proteins, is disrupted as DNA is unwound and copied. To understand how chromatin structure can be restored after DNA replication, it is important to understand how DNA replication affects chromatin proteins. Many chromatin proteins interact with DNA replication proteins, and these interactions are implicated in recruiting them to replicating DNA. Such chromatin proteins include Chromatin Assembly Factor 1 (CAF-1), the maintenance DNA methyltransferase (DNMT1), and histone modifying enzymes, all of which have been shown to associate with chromatin during DNA replication in cells through interactions with proteins at the replication fork. DNA replication proteins that interact with these chromatin proteins include the DNA polymerase processivity factor PCNA and the MCM replicative helicase[1–3]. These chromatin proteins may facilitate DNA replication itself, or be important for restoring chromatin structure on newly replicated DNA[4].

Proteins that are bound to chromatin throughout the cell cycle may also be stabilized on chromatin during the passage of the replication fork by interacting with replication proteins[5]. However, it is possible that some chromatin proteins have evolved to withstand passage of DNA replication forks.

Polycomb Group (PcG) proteins are essential regulators of development and differentiation that maintain gene repression by altering chromatin structure[6,7]. We previously found that the *Drosophila* PcG complex PRC1 remains bound to chromatin or DNA through replication in a cell-free system consisting of the SV40 large T-Antigen in conjunction with mammalian cell extracts[8]. Binding through DNA replication does not require nucleosomes but depends on self-association and DNA binding by the PSC subunit of PRC1[8,9]. PSC is bound throughout S-phase in cells, including to newly replicated DNA[8,9]. These experiments highlight the importance of the intrinsic self-association and DNA binding properties of PRC1 during DNA replication, but do not address whether interactions with eukaryotic replication proteins are also required. We therefore asked whether PRC1 could withstand passage of a T7 bacteriophage replication fork that does not use eukaryotic proteins.

Bacteriophage DNA replication systems have been pivotal to uncovering basic mechanisms of DNA replication[10]. This is because the fundamental processes of DNA replication, including template unwinding by protein helicases, and copying by DNA polymerases—are universal to DNA replication. Just two T7 bacteriophage DNA replication components are sufficient to unwind a double stranded DNA template and copy one strand: the gp4 replicative helicase[11], and the gp5 polymerase in a complex with the processivity factor, *E. coli* thioredoxin (gp5-trx)[12].

## Results

To replicate PRC1 bound DNA with the T7 system, a double stranded plasmid template with an entry site for the gp4 helicase was used (Fig. 1). The helicase unwinds the template in the 5′ to 3′ direction, allowing the T7

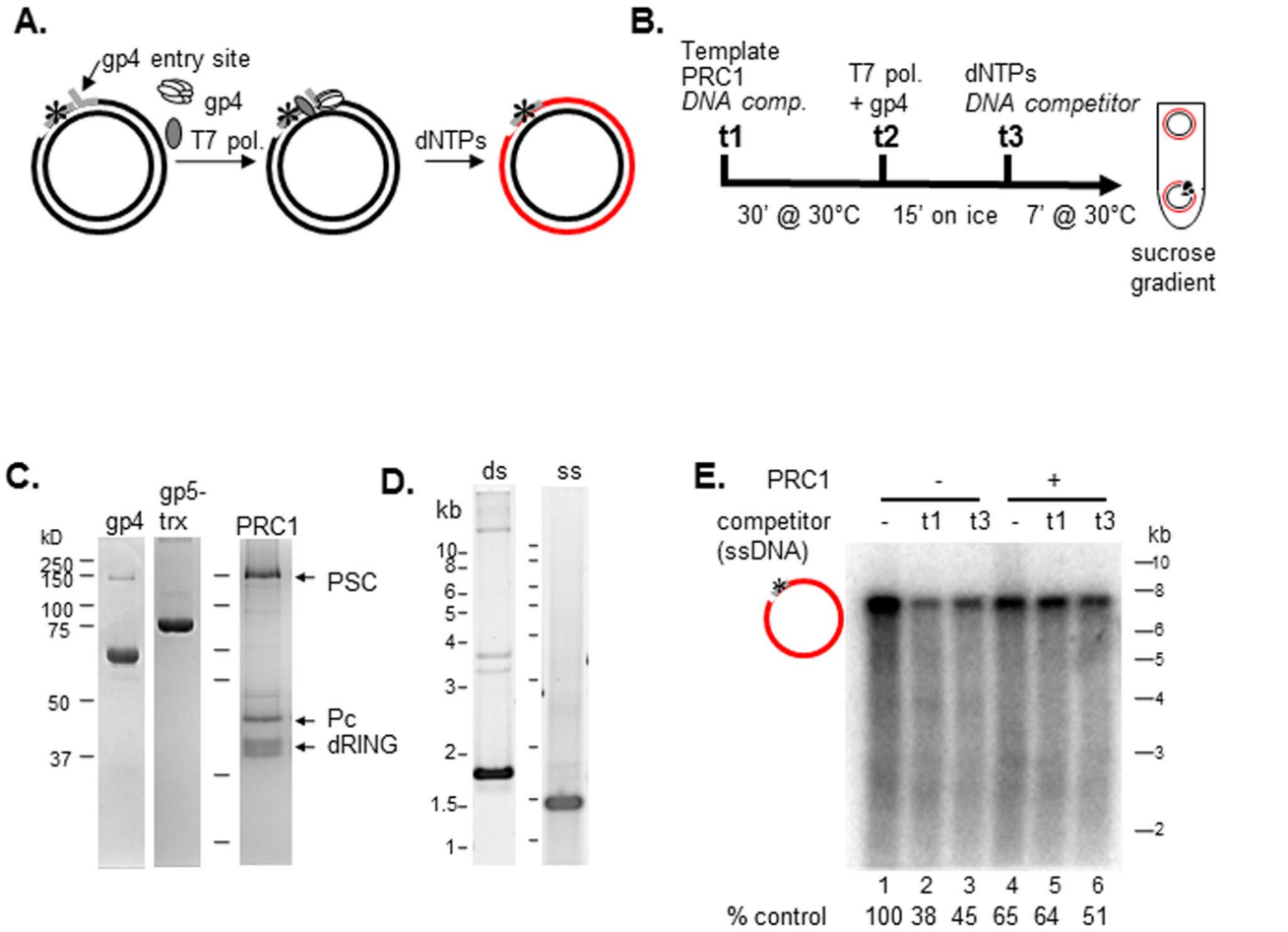


**Figure 1 | Establishment of T7 bacteriophage DNA replication system with PRC1.** A) Schematic of replication template; asterisk indicates radiolabel on primer. Extension of this primer by the T7 polymerase (gp5-trx) produces full length, radiolabelled products (red). B) Schematic of T7 replication experiments with PRC1. PRC1 is incubated with the template. Competitor DNA is added either with PRC1 (t1) or at the start of replication (t3). C) Colloidal Blue stained SDS-PAGE gel of gp4 helicase (~250 ng); gp5-trx (T7 polymerase-*E. coli* thioredoxin (note that trx is less than 14 kDa so is not visible on this gel) (~250 ng); PRC1 (~100 ng). D) Double stranded (ds) and single stranded (ss) DNA preparations of pUC19 circular DNA used as competitor were separated on 0.8% agarose, 1X TAE gels and stained with SYBR gold. E) Effect of competitor and PRC1 on DNA replication by T7 polymerase and gp4. Reactions were separated on alkaline agarose gels. Image shows phosphorimager scan of radiolabelled DNA. Red circle indicates the band that is the full length extension product as depicted in Fig. 1A. Full length replication products were quantified and expressed as percentage of control (lane 1). Where added (lanes 4–6), PRC1 was added at t1; if competitor is also added at this step, it is added just before addition of PRC1. Note that although competitor and PRC1 decrease replication, the products are still full length. In the experiments presented in Figures 2 and 3, we used reactions with competitor added during the replication step (t3) (without PRC1, as in lane 3) as a baseline so that comparable levels of signal are present in all reactions.

polymerase to copy the other strand. This strand displacement replication is analogous to leading strand replication, in that it involves unwinding of the DNA duplex, and synthesis of a continuous complementary DNA strand. T7 polymerase can carry out highly processive rolling circle replication under these conditions, producing long multimers of the template strand. To ensure that the plasmid templates are instead copied only once, a nick was introduced upstream of the helicase entry site to inhibit passage of the polymerase[13].

PRC1 was bound to DNA templates, which were then replicated with gp5-trx and gp4 (Fig. 1E, 2). To determine whether PRC1 is bound to DNA templates after replication, stopped reactions were sedimented through sucrose gradients. We previously showed that PRC1-bound plasmids sediment more rapidly in sucrose gradients than unbound plasmids[8]. We find that when PRC1 is bound prior to replication, the full-length replicated (radiolabeled) templates sediment near the bottom of the gradient, indicating that PRC1 is bound to the replicated DNA (Fig. 2B, C).

To determine if PRC1 remains bound through replication or is dislodged and rebinds, excess double or single stranded DNA competitor was added during the initial binding step (t1) or at the start of the replication reaction (t3) (Fig. 2). When competitor is added during the PRC1 binding step, it prevents PRC1 from binding to the template (Fig 2D, F; 3D, F). The replicated templates thus sediment near the top of the gradient. This indicates that the concentration of competitor is effective in capturing free PRC1 in the reaction. If the same amount of competitor DNA is added at the start of replication (t3), *after* PRC1 is bound to the template, the replicated templates sediment near the bottom of the gradient (Fig. 2E,G; 3E, G). This indicates that PRC1 is still bound to the replicated templates, even in the presence of competitor. We noticed that when competitor is added after PRC1 is bound to the template, the templates sediment more rapidly than when no competitor is added (Fig. 2C, E; 3C, E). Thus template-bound PRC1 may form additional contacts with the competitor plasmids, thereby creating more rapidly sedimenting

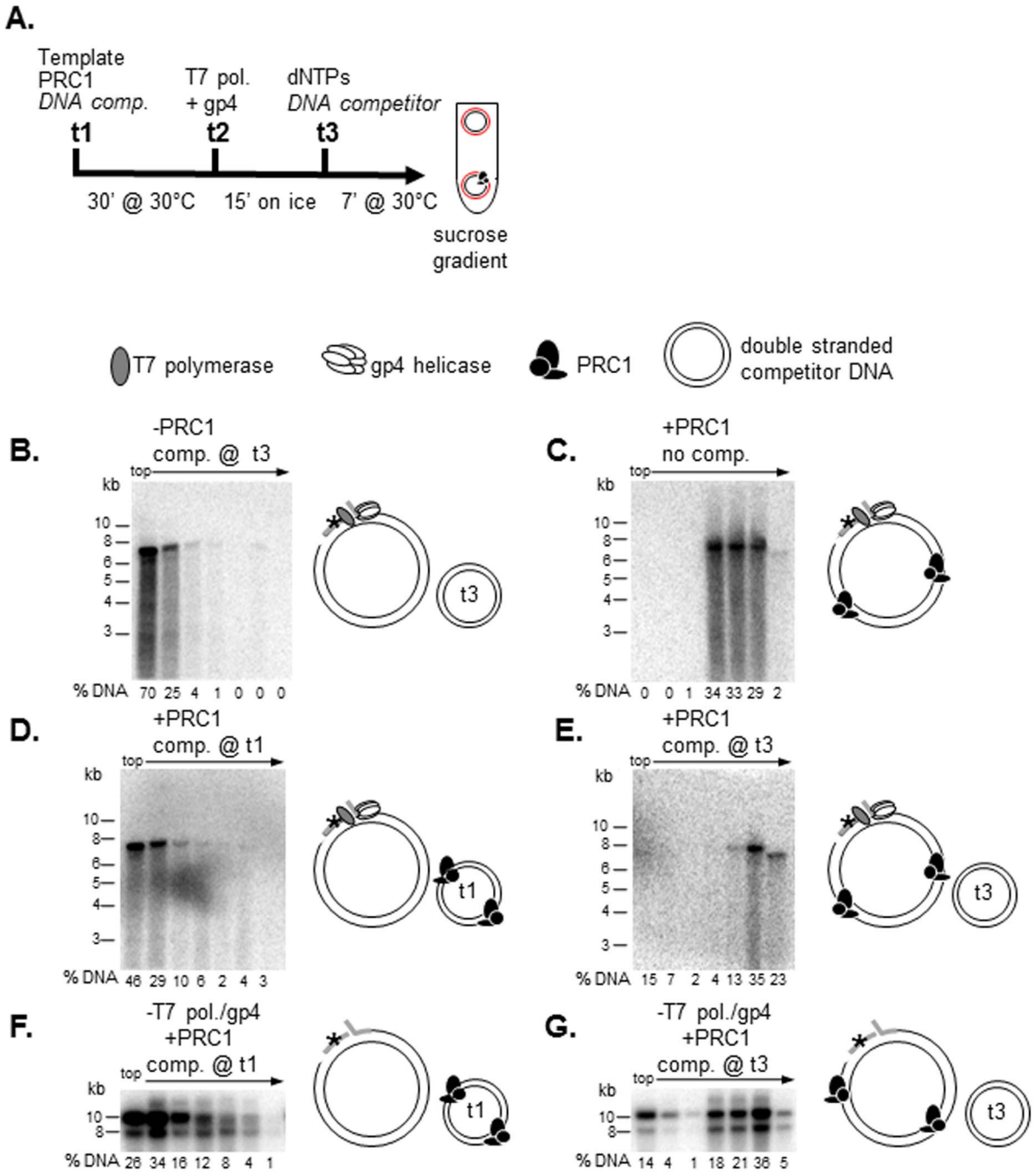


**Figure 2 | A PcG complex remains associated with templates through DNA replication with T7 bacteriophage proteins in the presence of double stranded DNA competitor.** A) Schematic of T7 replication experiments with PRC1. PRC1 is incubated with the template. Competitor DNA is added either with PRC1 (t1) or at the start of replication (t3). PRC1 bound and unbound templates are separated by sucrose gradient sedimentation. B–G)Phosphorimager scans of a T7 replication experiment. Reactions B–E were analyzed under denaturing conditions so bands show full length replication products; F and G (which are not replicated) were electrophoresed under native conditions since the labeled primer is not extended in the absence of T7 proteins.

complexes. We conclude that PRC1 remains bound to DNA during replication rather than being released and rebinding (Fig. 4).

## Discussion

These experiments demonstrate that interactions between PRC1 and DNA can withstand DNA unwinding and copying, the fundamental steps in DNA replication. Eukaryotic replication proteins are thus not required to tether PRC1 to the template through DNA replication. Importantly, it is still possible that PRC1 does interact with eukaryotic replication proteins, and that these interactions modulate its behavior during DNA replication. However, our results indicate that the ability to remain bound through the basic transactions of DNA replication is an intrinsic activity of PRC1 and does not depend on interactions with eukaryotic proteins. DNA binding and chromatin compacting activities of PRC1 are broadly conserved[14,15]; we hypothesize that PRC1 has evolved to withstand DNA replication to mediate epigenetic regulation. Recent work has highlighted the role of non-specific nucleic acid binding activity in tethering chromatin

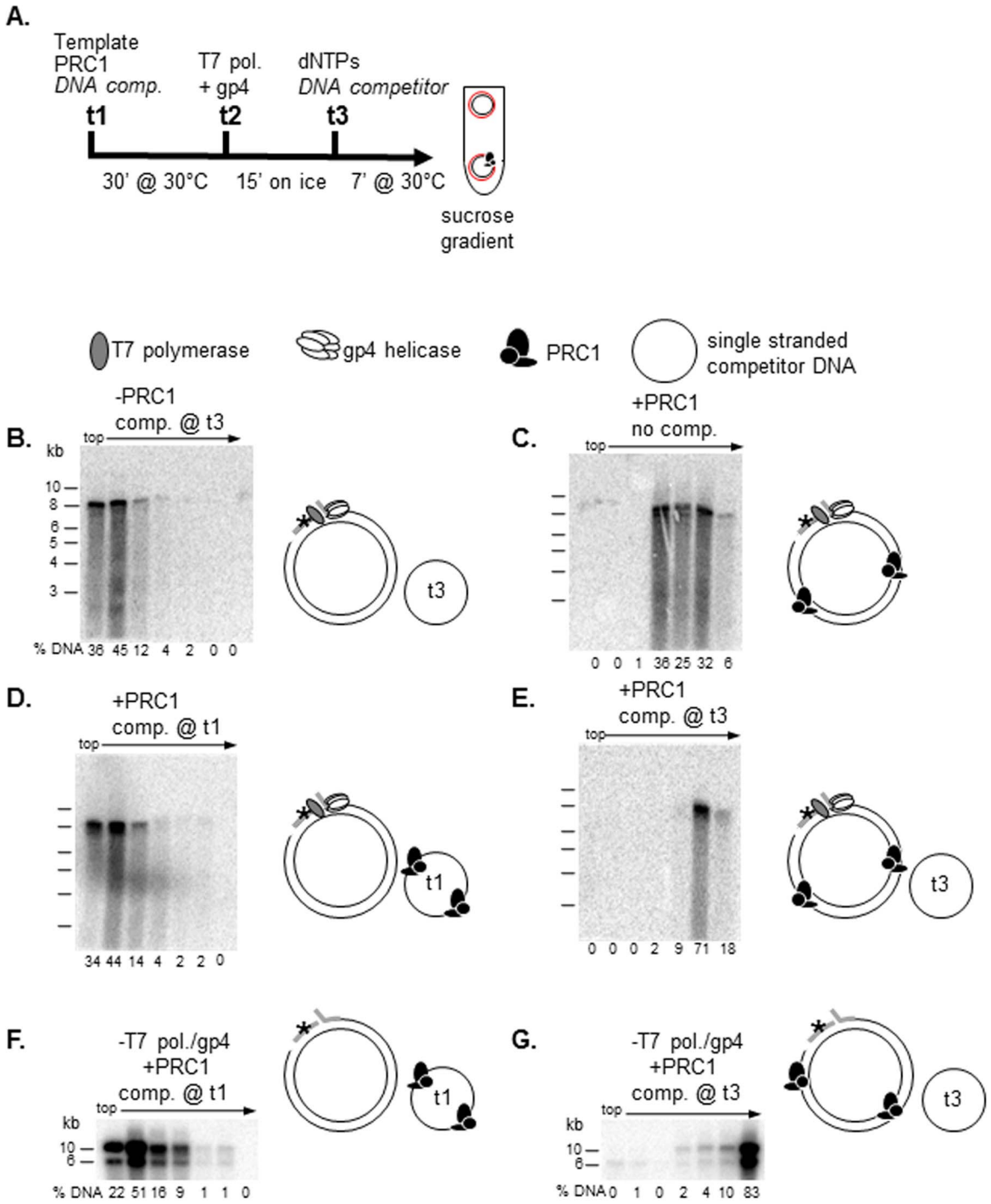


**Figure 3 | PRC1 remains bound through T7 DNA replication in the presence of single stranded DNA competitor.** A) Schematic of T7 replication experiments with PRC1. PRC1 is incubated with the template. Competitor DNA is added either with PRC1 (t1) or at the start of replication (t3). PRC1 bound and unbound templates are separated by sucrose gradient sedimentation (repeated from Fig. 2). B–G) Phosphorimager scans of a representative T7 replication experiment carried out with single stranded DNA competitor. Reactions were carried out as in Figure 2. Reactions B–E were analyzed under denaturing conditions so bands show full length replication products; F and G (which are not replicated) were electrophoresed under native conditions since the labeled primer is not extended in the absence of T7 polymerase.

proteins[16]. Our work suggests this activity may be especially important during DNA replication.

## Methods

**T7 template and competitor preparation.** The T7 template features a single stranded flap that serves as a 5′ entry site for the helicase gp4 which is adjacent to a labeled primer that is extended by T7 polymerase, and a nick created by the 5′ end of this primer which serves to terminate replication after a single round. To prepare the internally labeled oligonucleotide, oligonucleotide bml91 (GCGAATAATAATTT-TTTCACGTTGAAAATCTCCAAAAAAAAGG CTCCAAA) was 5′ end labeled with γ-ATP $P^{32}$, annealed to bml93 (AAAAATTA TTATTCGCAATTCCTTTA-GTTGT) and ligated to bml92 (GCGGAGTGAGAATA GAAAGGAACAA-CTAAAGGAATT). The internally-labeled and ligated product (bml91*92) was gel extracted from a 6% acrylamide 0.5X TBE/6 M Urea gel. Oligos bml75 (TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGGAGCCTTT AATTGTATCGGTTTATCAGCT) and bml91*92 were annealed to ssM13. T7 polymerase was used to extend from the 3′ end of bml75 around the ssM13 molecule

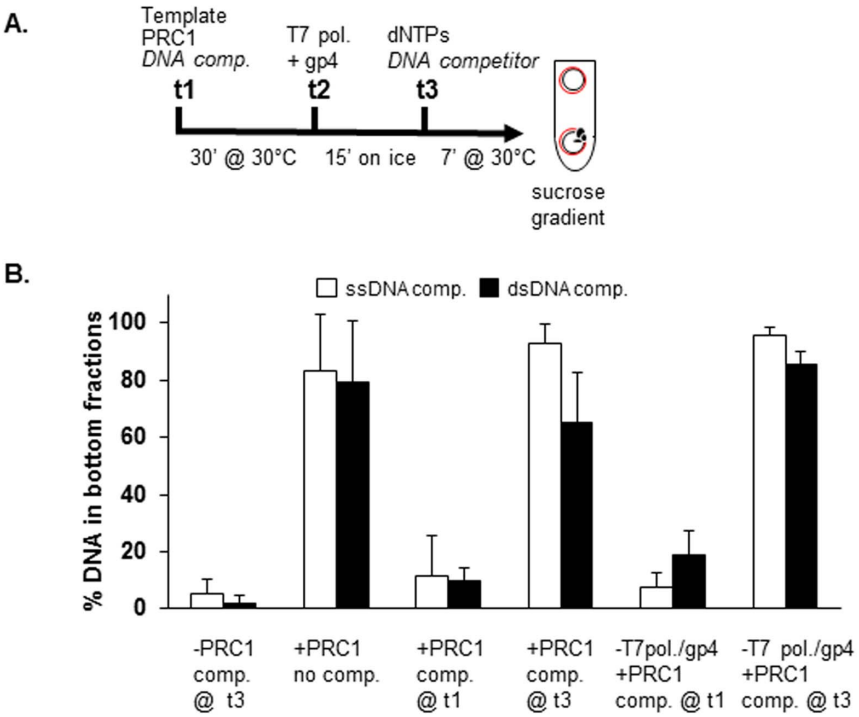


**Figure 4 | Summary of replication experiments demonstrating that PRC1 remains bound through DNA replication in the T7 bacteriophage system.** A) Schematic of T7 replication experiments with PRC1. PRC1 is incubated with the template. Competitor DNA is added either with PRC1 (t1) or at the start of replication (t3). PRC1 bound and unbound templates are separated by sucrose gradient sedimentation (repeated from Fig. 2). B) Graph shows the percentage of replicated DNA in the bottom four gradient fractions. Error bars indicate standard deviation.

to the 5′ end of bml91*92, creating a nicked template. The 5′ tail of bml75 is the entry point for the T7 helicase, gp4. Replication by T7 polymerase begins at the 3′ end of bml91*92.

Double stranded competitor DNA, dspUC19, was prepared by Qiagen Maxi prep. To prepare single stranded DNA from double stranded plasmids, dspUC19 was incubated with Nt.BspQI (New England Biolabs) to introduce a single nick. Exonuclease III (NEB) was incubated with the plasmid to degrade the nicked strand leaving sspUC19.

**T7 replication reactions.** 80 fmol of PRC1 were incubated with 5 fmol of template with or without competitor DNA (170 fmol sspUC19 or 225 fmol dspUC19) for 30 min. at 30°C in a final concentration of 2 mM $MgCl_2$, 0.05% NP40, 100 mM KCl, 7 mM HEPES pH 7.9 and a final volume of 6 µL. T7 polymerase-thioredoxin and gp4 helicase were added to a final concentration of 40 mM each and incubated for 10 min. on ice. The composition of the 10 µL reaction at this step is 5 mM $MgCl_2$, 0.05% NP40, 5 mM DTT and 50 mM HEPES pH 7.9. To start DNA replication, dNTPs and $MgCl_2$ were added along with competitor DNA in some cases. Reaction volumes are 14 µl at this stage and conditions were as stated above except for the addition of 0.5 µM dNTPs. Replication was carried out for 7 min. at 30°C. Reactions were stopped with a final concentration of 0.7 µM dideoxy-ATP (NEB). 2 µL of each reaction were removed to confirm replication. The rest was mixed with equal volume of 20% sucrose buffer and 10 µg BSA, sedimented through sucrose gradients and fractionated as described above. 25% of each fraction was electrophoresed on 0.8% agarose denaturing gels (25 mM NaOH, 2 mM EDTA). Reactions without replication where incubated with 1 µg/µL Proteinase K (PK) and 0.05% SDS at 50°C overnight and analyzed on 0.8% agarose, 1XTAE gels. Both native and denaturing gels were stained with SYBR gold and scanned on a Typhoon imager; before staining, denaturing gels were equilibrated with 1XTAE buffer. Gels were then incubated in 0.2 M HCl for 20 min. and denaturing solution (0.5 M NaOH, 1.5 M NaCl) for 30 min. DNA was transferred to HYBOND membranes under denaturing conditions and then exposed to a phosphorimager screen. Full-length replication product was quantified across the gradient fractions using ImageQuant. Signals from fractions 1–3 were pooled as the top of the gradient, while signals from fractions 4–6 plus the pellet were pooled as the bottom of the gradient.


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## Author contributions

B.M.L. designed, carried out, and analyzed the experiments, and wrote the paper. K.N.B. assisted with development of the replication assay and reagent preparation. S.G. and M.T. provided reagents and advice throughout the project. N.J.F. wrote the paper.

## Additional information

**Competing financial interests:** The authors declare no competing financial interests.



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