Restricting DNA Access Reveals Origin Licensing Dynamics and Sequence Dependence

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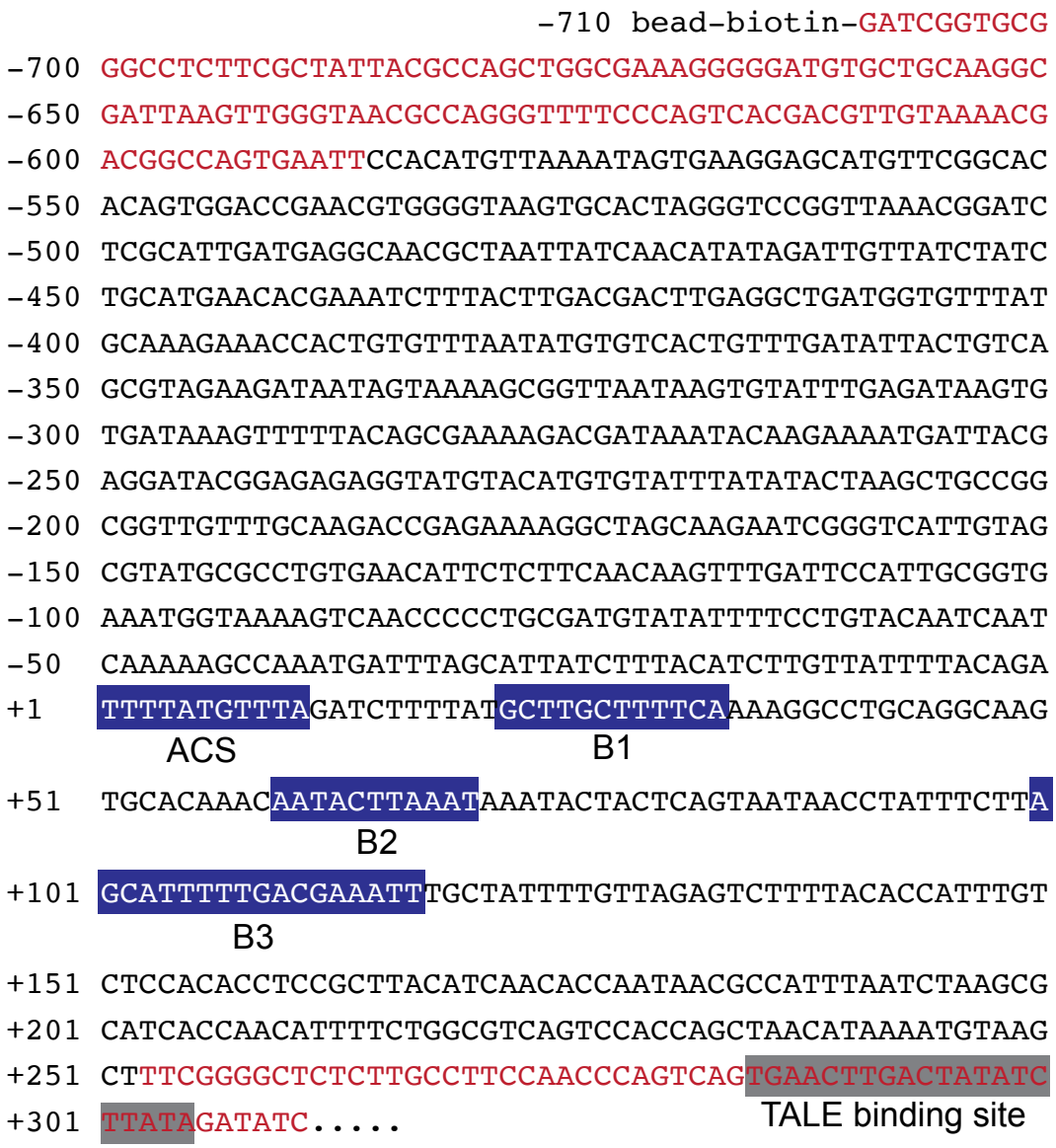
Supplemental Information

Figures S1 – S14

Tables S1-S3

References cited in Supplemental Information

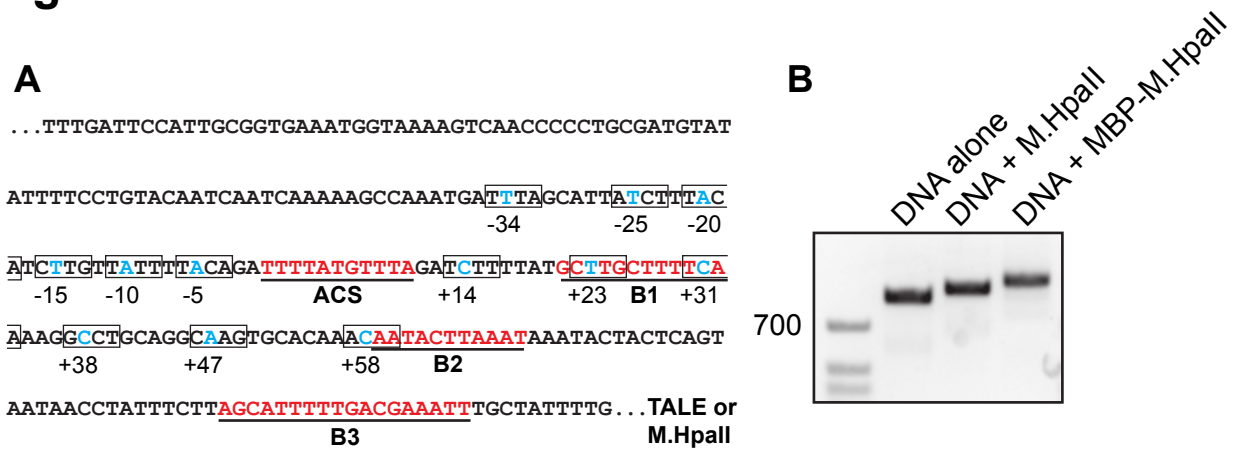
Figure S1



***ARS1* containing DNA template used in helicase-loading assays.**

Sequence of the T-rich strand of *ARS1* DNA used in helicase loading and OCCM formation assays. Black DNA seqeunce is *ARS1* DNA and the surrounding yeast DNA sequence. Red DNA sequence is non-yeast DNA. Blue boxes surround the indicated elements of *ARS1*. The gray box surrounds the TALE DNA binding site that is present in the experiments in Figures 1 and 2. This element is present in alternate locations in the templates used in Figures 4 and 5 (see Figure S6).

Figure S2

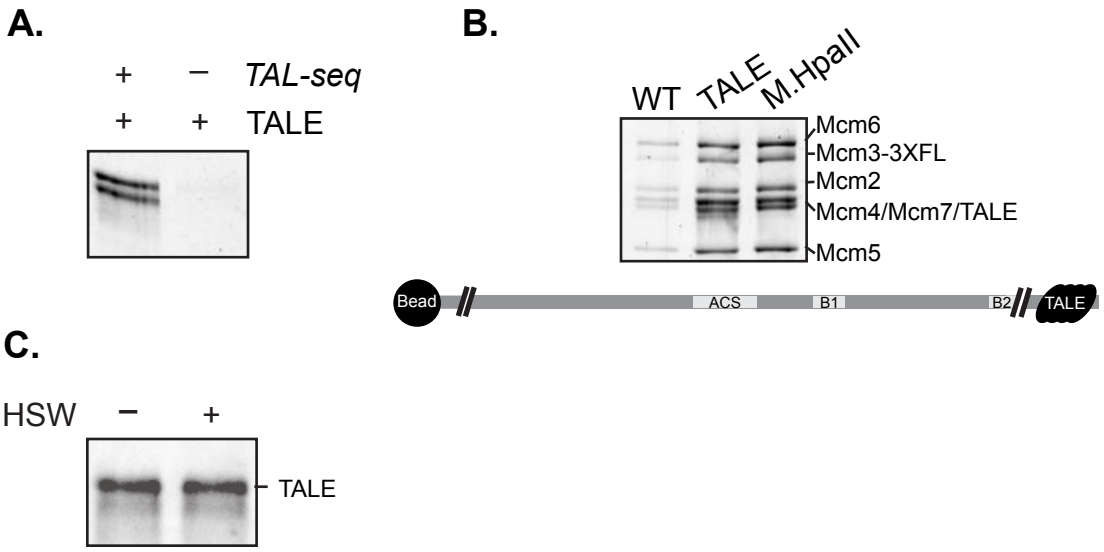


Location and extent of M.HpaII modification of *ARS1* DNA.

A) Sequence of the T-rich strand of *ARS1* DNA showing the locations of 5FdC-modified M.HpaII consensus sequence placement. Important features of *ARS1* are highlighted in red and labeled. Grey boxes identify sequences that were modified to the CCGG M.HpaII recognition motif. The number assigned to that location is written below each box. DNA residues in blue represent the exact location of the 5FdC to which M.HpaII was crosslinked.

B) M.HpaII crosslinking is quantitative. M.HpaII or MBP-M.HpaII was incubated with DNA containing a 5FdC-modified M.HpaII-recognition sequence and the resulting template was separated on a 1.5% agarose gel containing ethidium bromide. All detectable DNA template exhibits a shift when incubated with either M.HpaII or MBP-M.HpaII, indicating crosslinking of M.HpaII to the DNA was complete, as has been previously reported (1, 2).

Figure S3



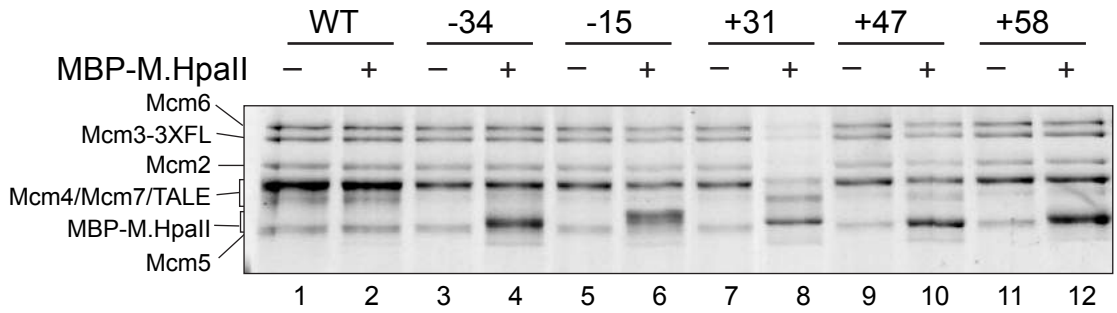
Characterization of TALE protein DNA binding.

A) TALE protein binding is sequence specific. DNA containing or lacking a TALE binding motif were incubated with purified TALE protein for 15 minutes, followed by a high-salt wash. Samples were run on an 8% SDS-PAGE gel and imaged by fluorescent protein staining. TALE binding was only observed when the binding sequence was included in the DNA template.

B) TALE or M.HpaII placement at the end of linear DNA templates increase amount of helicases detected. Substantially more helicases remain on the DNA when either TALE or M.HpaII is bound to the end of the template after either 20 min or 30 min of incubation with the helicase loading proteins. This is consistent with both complexes preventing loaded Mcm2-7 complexes from sliding off the end of the DNA during the helicase loading reaction, as has been previously observed (3, 4). A diagram of the location of the TALE binding within the bead-bound DNA is shown below.

C) TALE DNA binding is retained after a high-salt wash. After TALE protein was incubated with DNA containing its recognition motif for 15 minutes, free protein was either washed with a low-salt wash (left) or the same high-salt wash containing 500mM NaCl used to detect Mcm2-7 double-hexamer formation (right).

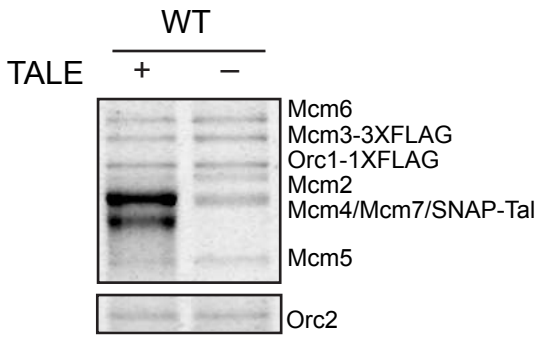
Figure S4



Increasing the size of the protein-DNA adduct did not alter the effect on helicase loading.

Maltose-binding protein was fused to the M.HpaII protein (M.HpaII = 40 kDa, MBP-M.HpaII = 80 kDa) and crosslinked to a subset of the 5FdC-modified *ARS1*-DNA templates. Increasing the size of the M.HpaII adduct did not change the locations where the M.HpaII did or did not affect helicase loading.

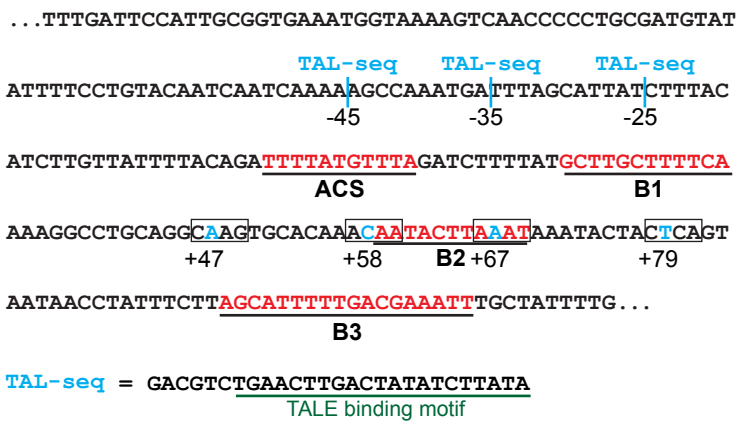
Figure S5



TALE binding to the free end of the DNA template does not increase ORC-Cdc6-Cdt1-Mcm2-7 (OCCM) complex formation.

An OCCM formation assay was performed on wild-type *ARS1*-DNA templates with and without a TALE protein bound to the free end of the DNA. Unlike the situation for the full helicase-loading assay, we observed no difference in the extent of OCCM intermediate formation in the presence or absence of the TALE protein.

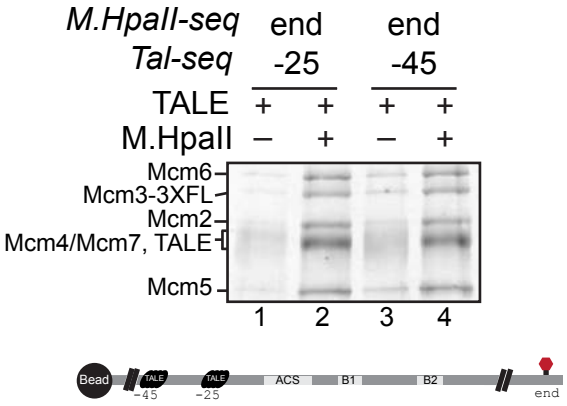
Figure S6



Locations of TALE-consensus sequence insertions

The sequence of the T-rich strand of *ARS1* is shown. Key sequence features of the origin are highlighted in red and labeled underneath. Locations that were changed to the M.HpaII sequence motif are boxed in grey. Locations where the TALE-binding sequence was inserted are shown by blue vertical lines. The insertion sequence included both the TALE-binding motif and an AatII restriction site and is shown below the *ARS1* sequence.

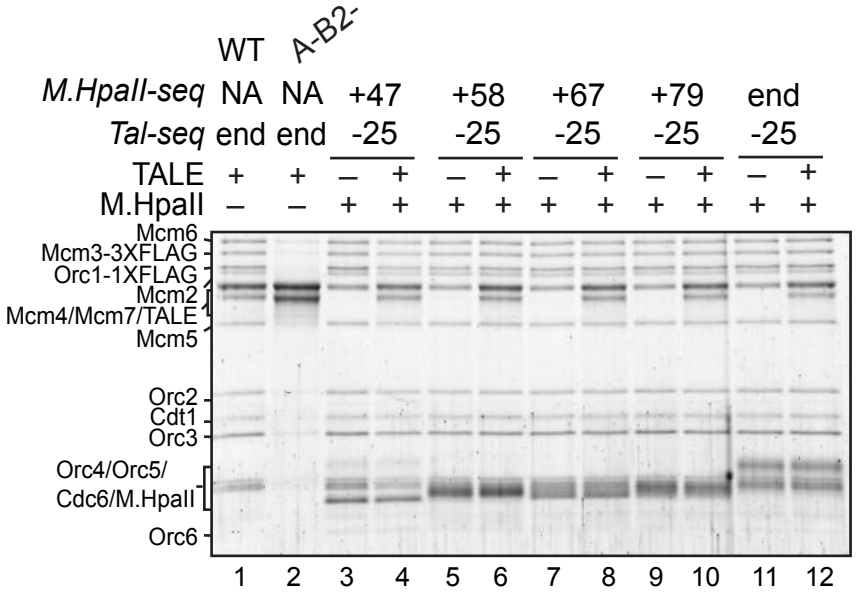
Figure S7



M.HpaII crosslinking inhibits Mcm2-7 sliding off DNA ends.

Helicase loading assays were performed in the presence of TALE protein binding with and without M.HpaII. The M.HpaII recognition sequence was located at the end of the template. Similar to what is observed in Figure S3B, Mcm2-7 signal is retained when M.HpaII cross-linked to the end of the DNA template.

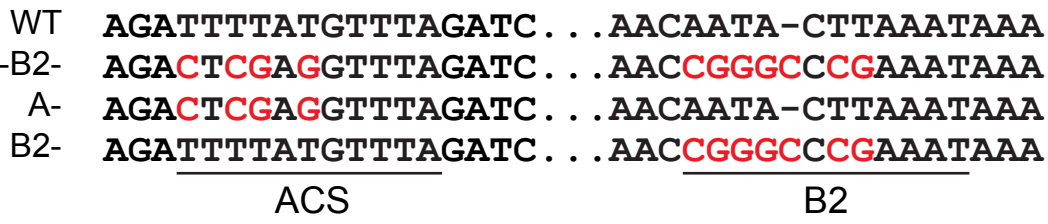
Figure S8



Proximal origin-flanking roadblocks did not affect OCCM formation.

OCCM formation was assessed using DNA templates in which a TALE binding motif was placed at the -25 location upstream of the ACS and M.HpaII crosslinked at five possible locations downsteam of the ACS. The sites of TALE-binding motif insertion and M.HpaII crossllinking are indicated above each template. The presense or absence of TALE protein or M.HpaII crosslinking is indicated. Lanes 1 and 2 show controls using wild-type (WT) ARS1 DNA or ARS1 DNA with a mutant in the ACS and B2 elements (A-B2-). These controle templates included a TALE-binding motif at the free-DNA end.

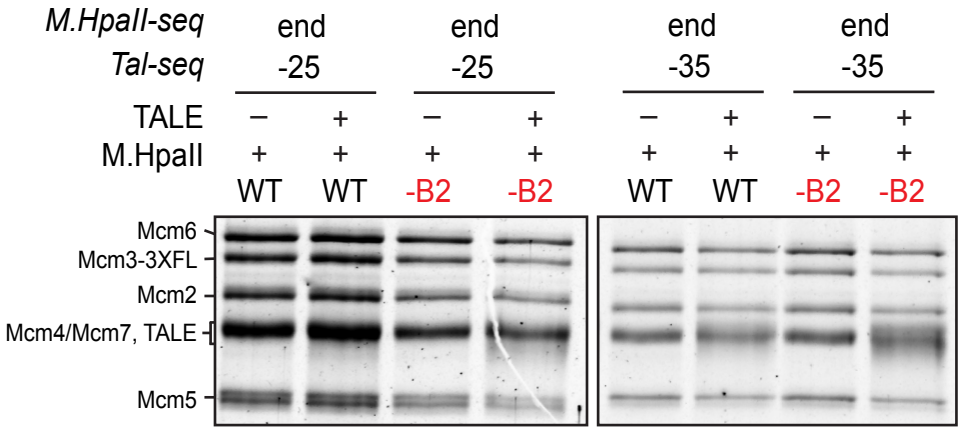
Figure S9



Mutant *ARS1*-DNA sequences tested.

The T-rich strand of WT and mutant ARS1 DNA templates used in this study are shown. Only the ACS and B2 elements are shown. The based mutated in each of the indicated mutations are shown in red.

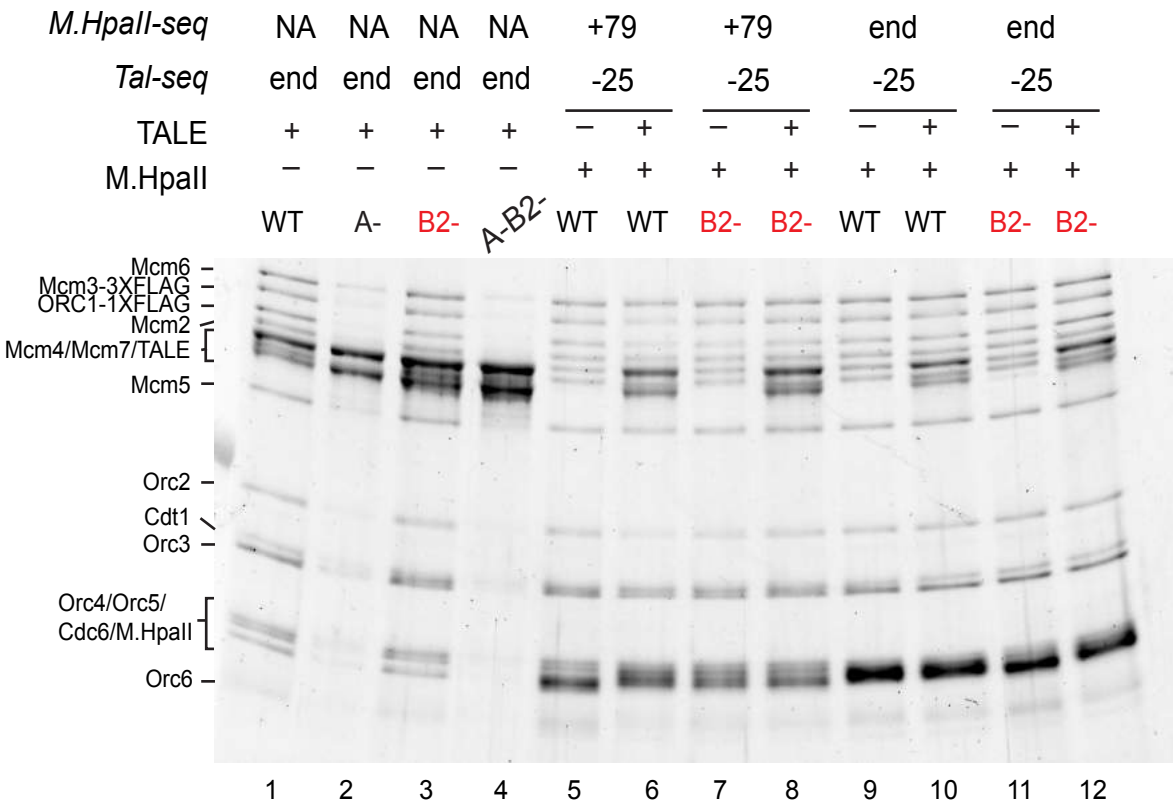
Figure S10



Distant origin-flanking roadblocks do not result in B2-dependent helicase loading.

The B2 element is not important for stable helicase loading when the TALE protein is bound at -25, or -35 and the M.HpaII is crosslinked at the free end of template (~+280). The sites of TALE-binding motif insertion and M.HpaII crossllinking are indicated above each template. The presense or absence of TALE protein or M.HpaII crosslinking is indicated. The presence of a wild-type (WT) or mutant (B2-) B2 motif in the template is indicated

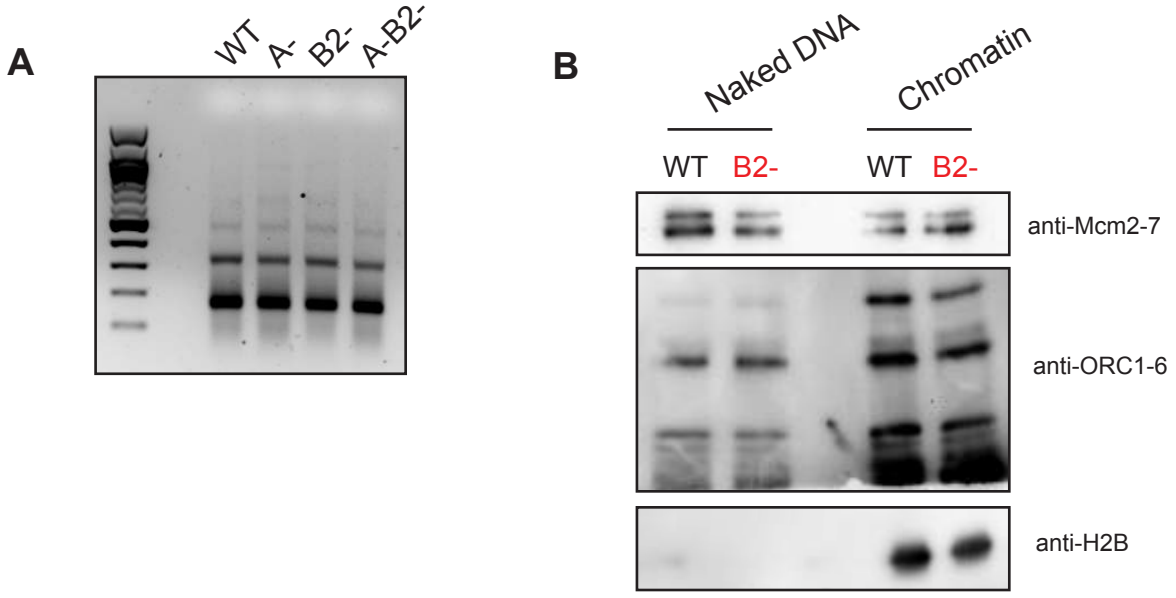
Figure S11



OCCM formation is not inhibited by mutation of B2 in the presence of origin-proximal roadblocks

OCCM formation assays were performed on a subset of templates used in Figure 5C. The sites of TALE-binding motif insertion and M.HpaII crossllinking are indicated above each template. The presense or absence of TALE protein or M.HpaII crosslinking is indicated. Lanes 1-4 show control reactions with the indicated ARS1 mutations (see Fig. S9) and these templates include a TALE-binding motif at the free end of the DNA template. Although clear defects in OCCM formation were observed when the ACS was mutated, no detectable changes in OCCM formation were observed in either WT or B2- templates with single or origin-proximal flanking roadblocks.

Figure S12

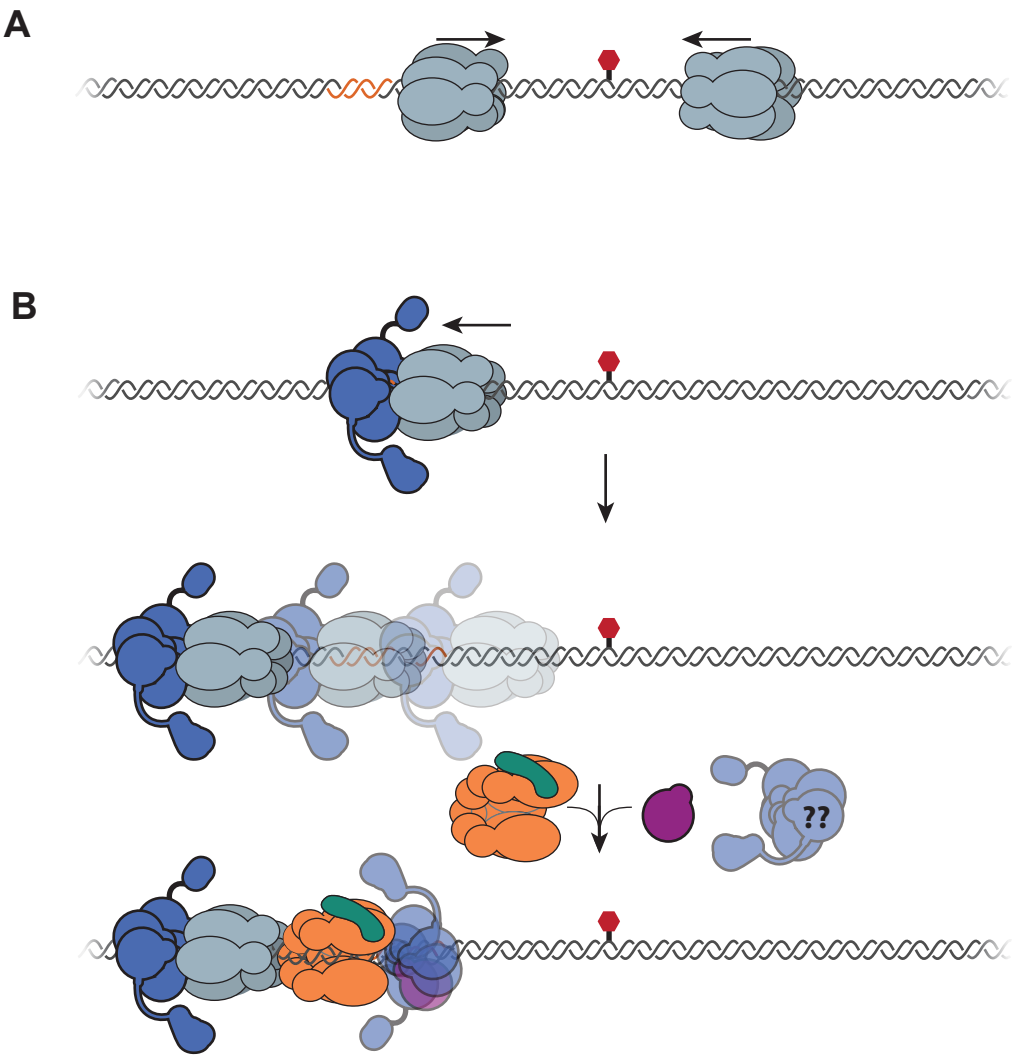


Origin mutations do not effect the extent of nucleosome assembly or OCCM formation in the presence of nucleosomes.

A) *ARS1*-DNA mutations do not alter the extent of ordered nucleosome assembly on *ARS1* DNA. The indicated wild-type and mutant *ARS1*-DNA templates were assembled into nucleosomes and treated with limiting amounts of MNase.

B) OCCM formation is similar on wild-type and B2 mutant ARS1-DNA templates. OCCM formation was tested using the indicated templates either as naked DNA or after assembly of the DNA into nucleosomes. Immunoblots comparing Mcm2-7 association, ORC association and histone H2B association are shown.

Figure S13



Models for sliding of Mcm2-7 complexes on origin DNA after initial DNA association

A. Movement of helicases after initial DNA association as proposed by Coster and Diffley (2017) (5). Two Mcm2-7 helicases are recruited independently at two separate locations, then slide via an active mechanism to form a double hexamer. Based on current understanding of the direction of the Mcm2-7 complex, such an active mechanism requires the Mcm2-7 complexes to slide towards the M.HpaII adduct pictured.

B. Sliding of the helicase based on data presented in this manuscript. For successful double-hexamer formation, a helicase-loading intermediate must slide away from the M.HpaII roadblock, leaving enough space for loading of the second Mcm2-7 hexamer.

Figure S14

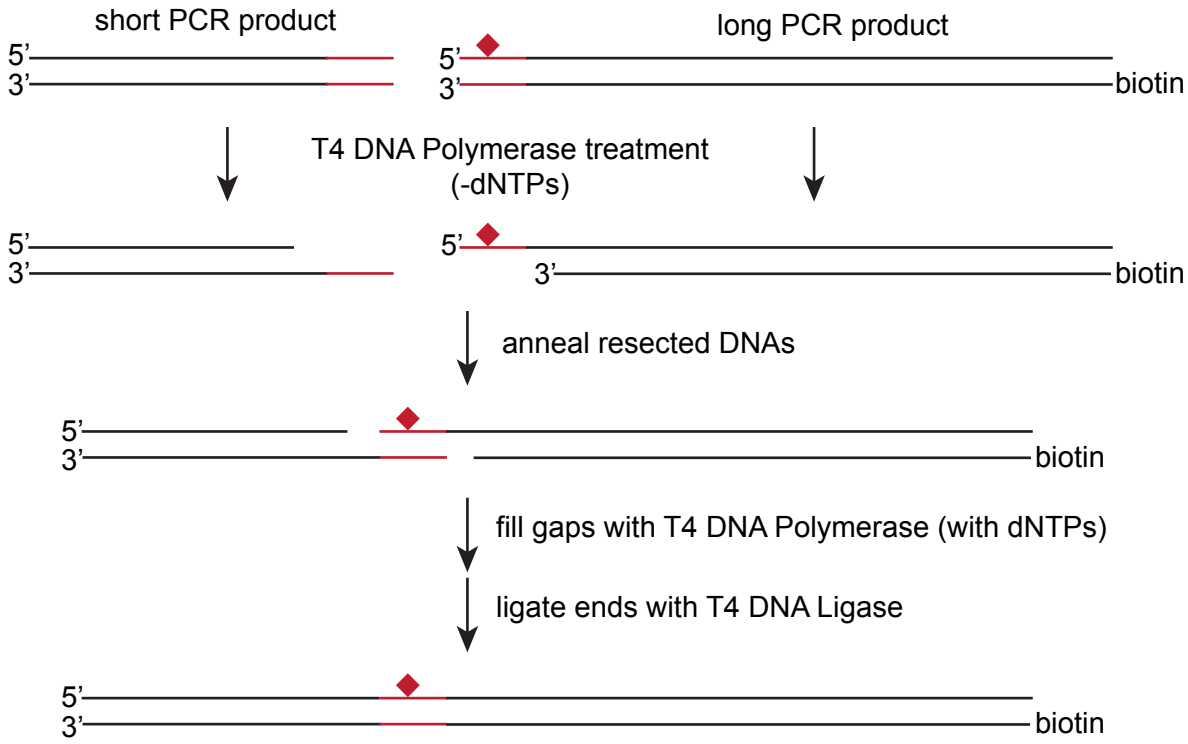


Illustration of method to produce 5FdC modified ARS1-DNA templates.

The steps of producing ARS1-DNA templates with a single M.HpaII recognition motif including 5FdC is illustrated. The region of homology between the long and short PCR products includes the M.HpaII site in both products and is shown in red. Only the long PCR product includes a single site of 5FdC-modified DNA. The site of biotin attachment to the DNA is also indicated.

Table S1: Strains used in this study

Strain Name	Strain use	Strain details	Study?
ySK100	ORC expression strain	*MATa ade2-1 ura3-1 his3-11,15 can1-100 bar1::HisG orc1Δ::HisG pep4Δ::KanMX LEU2::ORC1 TRP1::p404-GAL1-10-ORC3,4 LYS2::plys2-GAL1-10-ORC2,5 HIS3::p403-GAL1-10-1xFlagORC1,6*	(6)
yST144	Mcm2-7/Cdt1 expression strain	*MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-1 can1-100 bar1Δ::HisG lys2Δ::HisG pep4Δ HIS3::pSKM004 (GAL1,10-MCM2, Flag-MCM3) URA3::pALS1 (GAL1,10-Cdt1, GAL4) LYS2::pSKM002 (GAL1,10-MCM4, MCM5) TRP1::pSKM003 (GAL1,10-MCM6, MCM7)*	(8)

Table S2: Oligonucleotides used in this study

Oligo Name	Oligo Sequence (5'-->3')	Study?
+79 FWD B2-	GCCCGAAATAAATACTACCGGGTAATAACCTATTTCTTAGC	This Study
+79 REV B2-	GAAATAGGTTATTACC(5FdC)GGTAGTATTTATTTCGGGCCCGG	This Study
biopUC19 seqA	(5biodT)GATCGGTGCGGGCCTCTTCGC	(6)
pUC19 seq A	GATCGGTGCGGGCCTCTTCGC	(6)
SNAP-Tal seqB	GATATCTATAAGATATAGTCAAGTTCACTGACTGGGTTGGAAGGC	This Study
Nco Pri1	CTGACTGGGTTGGAAGGCAAGAGAGCC	This Study
+79 REV	GAAATAGGTTATTACC(5FdC)GGTAGTATTTATTTAAGTATTGTTTGTGC	This Study
+67 REV	CTGAGTAGTATTTC(5FdC)GGAAGTATTGTTTGTGCACTTG	This Study
+58 REV	GTATTTATTTAAGTAC(5FdC)GGTTGTGCACTTGCCTGC	This Study
+47 REV	AGTATTGTTTGTGCAC(5FdC)GGCCTGCAGGCCTTTTG	This Study
+38 REV	GTGCACTTGCCTGCC(5FdC)GGCTTTTGAAAAGCAAGCATAAAAG	This Study
+23 REV	GCAGGCCTTTTGAAAAGC(5FdC)GGCATAAAAGATCTAAACATAAAATCTG	This Study
+14 REV	GAAAAGCAAGCATAAC(5FdC)GGTCTAAACATAAAATCTGTAAAATAACAAG	This Study
-5 REV	GATCTAAACATAAAATCC(5FdC)GGAAATAACAAGATGTAAAGATAATGC	This Study

-10 REV	ACATAAAATCTGTAAC(5FdC)GGACAAGATGTAAAGATAATGCTAAATC	This Study
-15 REV	TCTGTAAAATAAC(5FdC)GGATGTAAAGATAATGCTAAATCATTTG	This Study
-20 REV	TGTAAAATAACAAGAC(5FdC)GGAAGATAATGCTAAATCATTTGGC	This Study
-25 REV	AATAACAAGATGTAAC(5FdC)GGAATGCTAAATCATTTGGCTTTTTG	This Study
-34 REV	ATGTAAAGATAATGCC(5FdC)GGTCATTTGGCTTTTTGATTGATTG	This Study
+58 FWD	CAGGCAAGTGCACAACCGGTACTTAAATAAATACTACTCAGTAATAACC	This Study
+47 FWD	CAAAAGGCCTGCAGGCCGGTGCACAAACAATACTTAAATAAATAC	This Study
+38 FWD	GCTTTTCAAAAGCCGGGCAGGCAAGTGCAC	This Study
+23 FWD	GATCTTTTATGCCGGCTTTTCAAAAGGCCTGC	This Study
+14 FWD	GATTTTATGTTTAGACCGGTTATGCTTGCTTTTCAAAAGG	This Study
-5 FWD	ATCTTTACATCTTGTCCGGTTACAGATTTTATGTTTAGATCTTTTATG	This Study
-10 FWD	ATCTTTACATCTTGTCCGGTTACAGATTTTATGTTTAGATCTTTTATG	This Study
-15 FWD	AGCATTATCTTTACATCCGGTTATTTTACAGATTTTATGTTTAGATC	This Study
-20 FWD	ATTTAGCATTATCTTCCGGTCTTGTTATTTTACAGATTTTATGTTTAG	This Study
-25 FWD	CAAATGATTTAGCATTCCGGTTACATCTTGTTATTTTACAGATTTTATG	This Study
-34 FWD	CAAAAAGCCAAATGACCGGGCATTATCTTTACATCTTGTTATTTTAC	This Study
Tal Insert -25	GTAAAATAACAAGATGTAAAGTGAACTTGACTATATCTTATAATAATGCTAAATCATTTG	This Study
Tal Insert -35	GTAAAGATAATGCTAAAGACGTCTGAACTTGACTATATCTTATATCATTTGGCTTTTTGATTG	This Study
Tal Insert -45	GATAATGCTAAATCATTTGGCTGAACTTGACTATATCTTATATTTTTGATTGATTGTAC	This Study

Table S3: Plasmids used in this study

Plasmid Name	**Construct contains**	**Study?**
pM15MH	M.HpaII Expression plasmid	(2)
pM15MH-MBP	M.HpaII with a C terminal MBP tag	This study
pSNAP-Tale	TALE protein with SNAP tag	This study
pUC19 ARS1	*ARS1* WT	(7)
pUC19 ARS1 NcoI	*ARS1* WT with flanking NcoI sites	This study
pUC19 ARS1-A-	*ARS1* with a mutation in the A element	This study
pUC19 ARS1-B2-	*ARS1* with a mutation in the B2 element	This study
pUC19 ARS1-A-B2-	*ARS1* with mutations in the A and B2 elements	(7)
pUC19 ARS1 -25 Talseq	ARS1 WT with a TALE recognition sequence at -25	This study
pUC19 ARS1 -35 Talseq	ARS1 WT with a TALE recognition sequence at -35	This study
pUC19 ARS1 -45 Talseq	ARS1 WT with a TALE recognition sequence at -45	This study
pUC19 ARS1 -25 Talseq B2-	ARS1 B2- with a TALE recognition sequence at -25	This study
pUC19 ARS1 -35 Talseq B2-	ARS1 B2- with a TALE recognition sequence at -35	This study
pUC19 ARS1 -45 Talseq B2-	ARS1 B2- with a TALE recognition sequence at -45	This study
pARS1 Nco/Nco WT	ARS1 WT construct for nucleosomal templates	(6)
pARS1 Nco/Nco A-	ARS1 A- construct for nucleosomal templates	(6)
pARS1 Nco/Nco B2-	ARS1 B2- construct for nucleosomal templates	(6)
pARS1 Nco/Nco A-B2-	ARS1 A-B2- construct for nucleosomal templates	(6)

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