Supplemental Data

Mitotic Remodeling of the Replicon and Chromosome Structure

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Supplemental References

Almouzni, G., and Mechali, M. (1988). Assembly of spaced chromatin promoted by DNA synthesis in extracts from Xenopus eggs. EMBO J. 7, 665–672.

Coue, M., Kearsey, S.E., and Mechali, M. (1996). Chromotin binding, nuclear localization and phosphorylation of Xenopus cdc21 are cell-cycle dependent and associated with the control of initiation of DNA replication. EMBO J. 15, 1085–1097.

Ioudinkova, E., Petrov, A., Razin, S.V., and Vassetzky, Y.S. (2005). Mapping long-range chromatin organization within the chicken alpha-globin gene domain using oligonucleotide DNA arrays. Genomics 85, 143–151.

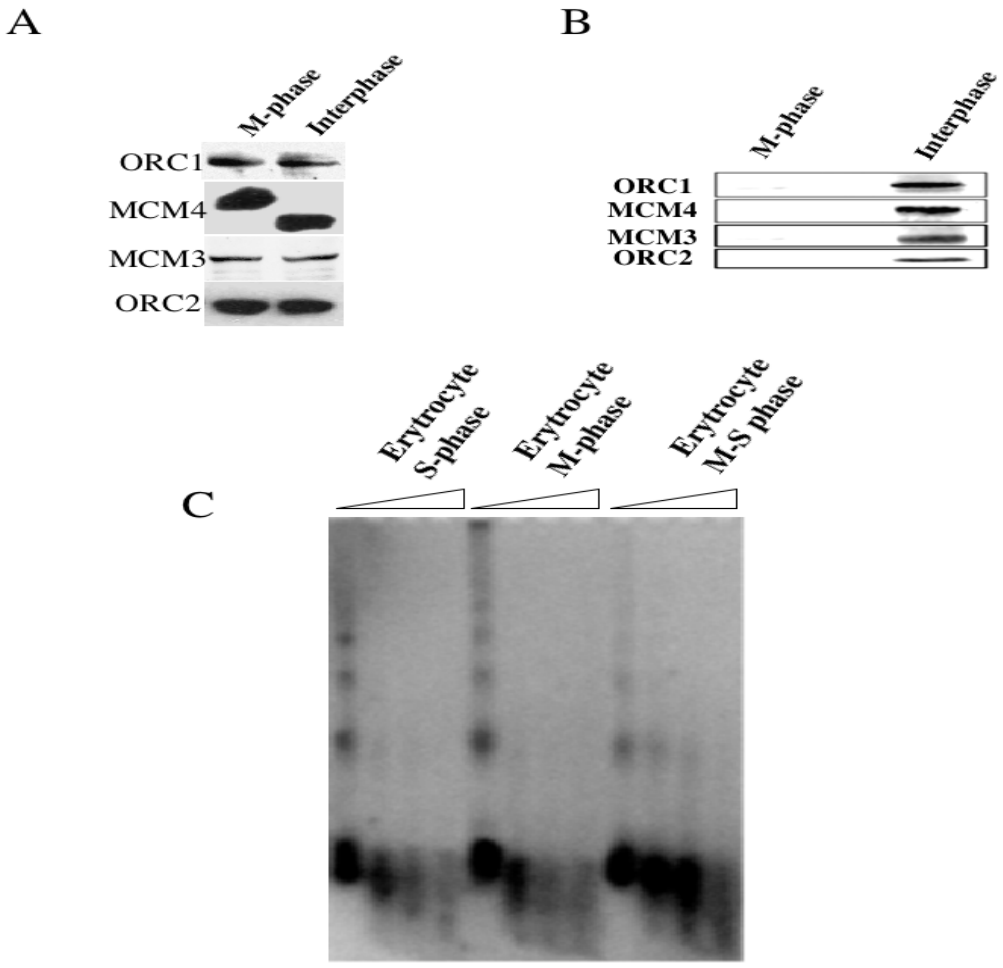

Figure S1. Pre-RC Proteins and Nucleosome Assembly in M Phase

(A) One microliter of extract was loaded onto a 10% SDS PAGE and transferred for immunoblotting with ORC1, ORC2, MCM3 and MCM4 antibodies. Note the dephosphorylation of MCM4 at mitosis exit, as previously described (Coue et al., 1996).

(B) Chromatin assembled in M-phase or S-phase extract was purified as described in Experimental Procedures and analyzed for ORC1, ORC2, MCM4, and MCM3 binding.

(C) Nucleosome spacing assay: Chromatin was purified from erythrocytes, incubated either in S-phase extract or for 45 min in M-phase extract before S-phase triggering with $CaCl_2$, and resuspended in micrococcal digestion buffer containing 0.3% Triton X-100 and 3 mM $CaCl_2$. Chromatin digestion was initiated by adding 60 U micrococcal nuclease, as described (Almouzni and Mechali, 1988), and DNA products were analyzed by 1.8% agarose gel electrophoresis. A ladder of nucleosomes was observed in both cases.

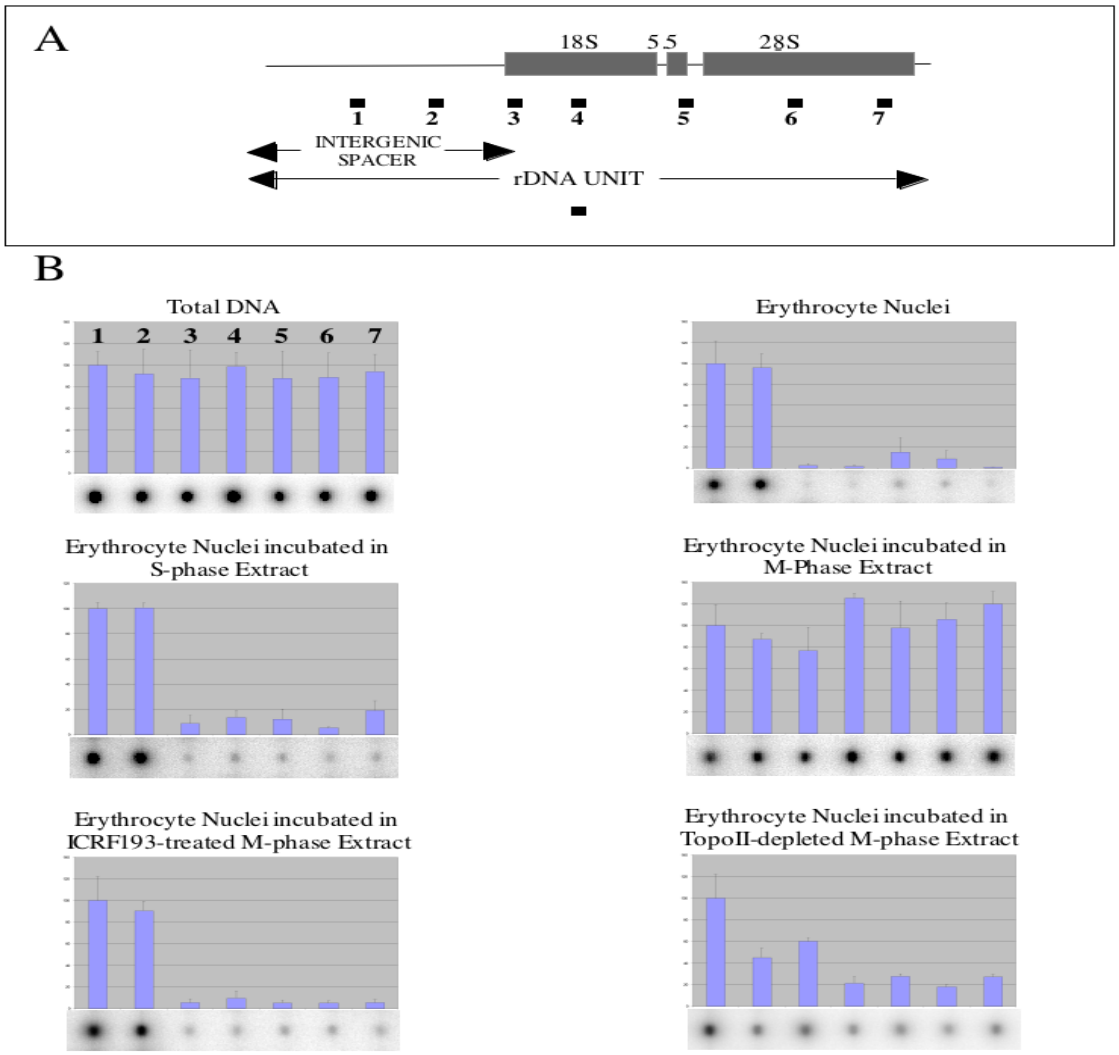

Figure S2. Chromatin Organization in the rDNA Domain Revealed Using DNA Arrays

A recently-developed method for mapping the interactions of DNA with the nuclear matrix based on oligonucleotide DNA arrays (Ioudinkova et al., 2005) was used to confirm the results presented in Figure 4.

Methods: Oligonucleotide DNA Array

The array was devised to use the complete Xenopus rDNA sequence, assembled from entries X05264 and X02995 (Genbank) and comprising 11505 base pairs. The micro-array consisted of seven oligonucleotides spaced approximately 1500 bp apart (see following Table) and possessing similar sizes (25-30 bp) and annealing temperatures (60±1°). The first two oligonucleotides covered the intergenic spacer and the other five spanned the 40S transcript.

The oligonucleotides were slot-blotted onto Zeta-probe GT filters in 0.4 NaOH and fixed by baking at 80°C for 30 min. Each filter contained the array in duplicate. The hybridization was carried out at 58°C in modified Church buffer (0.5 M phosphate buffer pH 7.2, 7% SDS, 10 mM EDTA) overnight. The blot was subsequently washed in 2x SSC, 0.1% SDS twice for 5 min, and then in 1x SSC, 0.1% SDS, twice for 10 min and exposed on a

PhosphoImager. All experiments were done in duplicate. The data has been normalized versus an internal control (oligonucleotide No.1).

Position	Oligonucleotide	Size, nt	Annealing Temperature, °C
1476	GGAGAGGTAGAGACAAGACAGAGGC	25	60.3
2723	GGGCGAAGAAAACCGGGAGAAATAC	25	60.8
4136	GAGAGAAAGACGGAAAGAAAGGAGAGTAG	29	60
5279	CATTCGTATTGTGCCGCTAGAGGTG	25	60.7
7181	CCACGACTCAGACCTCAGATCAGAC	25	60.8
8829	GTAACAACTCACCTGCCGAATCAACTA	27	60.3
10262	CTGTGAAGAGACATGAGAGGTGTAGGATAA	30	60.9

Results

(A) rDNA gene domain. Numbered blocks indicate the positions of the oligonucleotides within the array. The graphs represent the hybridization ratios of nuclear matrix-associated DNA normalized against an internal oligonucleotide no. 1. The average of two independent experiments is presented (B). Total Xenopus DNA hybridized equally to the rDNA array, suggesting that the chosen oligonucleotides do not contain external DNA repeats. Nuclear matrix DNA from erythrocytes only hybridized to the two oligonucleotides corresponding to the intergenic spacer, confirming the results of Figure 4. Incubation of erythrocyte nuclei in S-phase extract did not alter the hybridization pattern. Incubation of erythrocyte nuclei in mitotic extracts led to a hybridization pattern similar to that of total DNA, suggesting a lack of specific locus attachment sites. Inhibition of DNA topoisomerase II in the M-phase extract or, to a lesser extent, depletion of DNA topoisomerase II with specific antibodies, maintained the original organization of the rDNA gene domain.

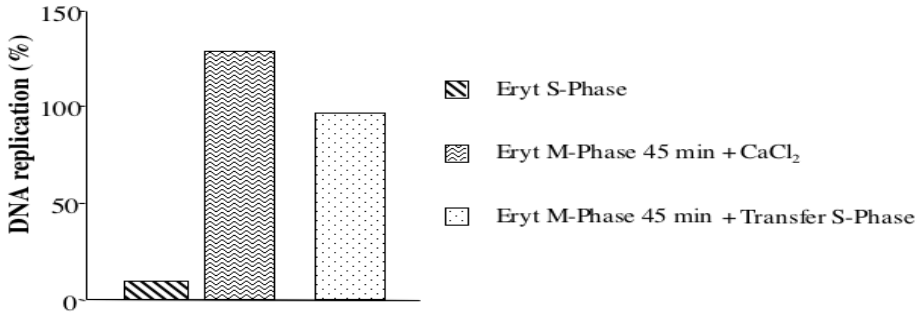

Figure S3. Erythrocyte Remodeling Does Not Occur during the Transition from M to S Phase

Sperm nuclei and erythrocytes nuclei were incubated for 45 min in M-phase extract and chromatin purified without previous activation of the M-phase extract. It was then directly transferred to S-phase extract. DNA replication was measured by a 3 hour incubation in S-phase extract in the presence of ^{32}PαdCTP.

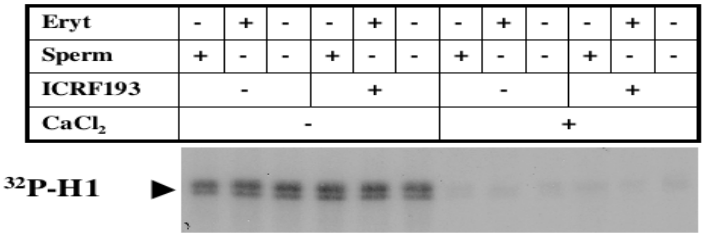

Figure S4. Topo II Inhibition by ICRF193 in M Phase Extract Does Not Induce Exit from Mitosis

Sperm or erythrocyte nuclei were incubated in M-phase extract with or without 50 µg/ml ICRF193 for 45 min. Mitotic exit was then triggered by adding 1 mM $CaCl_2$ and monitored 30 min later by measuring H1 kinase activity.

H1 kinase assays were performed in kinase buffer (250 mM sucrose, 10 mM MgCl2, 50 mM KCl, 10 mM Hepes, pH 7.7), supplemented with 40 µM cold ATP, 0.2 µCi of α-32 ATP, 0.2mg/ml histone H1 (Sigma), and 50mM β-glycerophosphate. Ten microliters of the above mixture was supplemented with 0.01 µl of the extract to be assayed and incubated at room temperature for 5 min, and the reaction was stopped by the addition of 10 µl of 2X Laemmli protein gel loading buffer. Reaction products were separated by PAGE, and the phosphorylated histone H1 was detected by autoradiography.

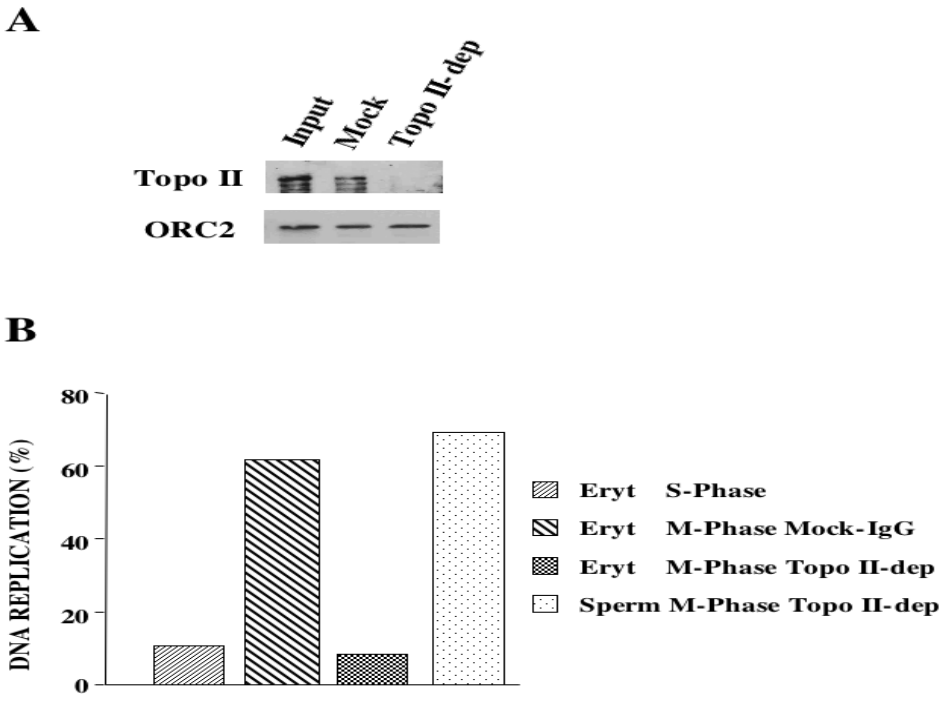

Figure S5. Topo II Depletion in M Phase Extract Prevents Mitotic Remodeling of Erythrocyte Nuclei

Depletion of TopoII in M-phase was carried out using an anti-TopoII polyclonal antibody kindly provided by Dr. Bogenhagen. Depletion was monitored by immunoblot (A). Erythrocyte nuclei and sperm nuclei were incubated in a depleted M-phase extract for 45 min and S-phase was triggered by addition of $CaCl_2$. DNA replication was measured after 3 hours of incubation. A mock depletion was also performed using a non-specific rabbit IgG.

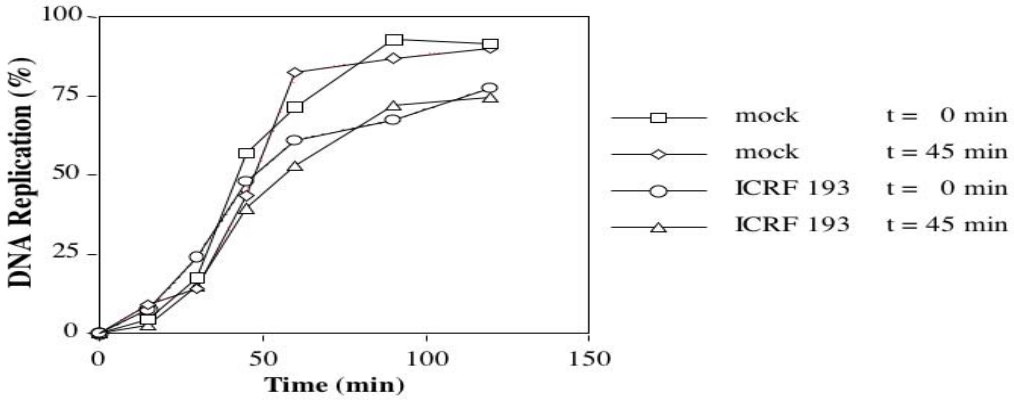

Figure S6. Topo II Inhibition of S Phase Extracts by ICRF193 Does Not Interfere with DNA Replication

Sperm chromatin was incubated in S-phase extract in the presence of 50 μg/ml ICRF193 at the beginning of the reaction or after 45 min (during the elongation phase). DNA replication was monitored by TCA precipitation of the replicated DNA.

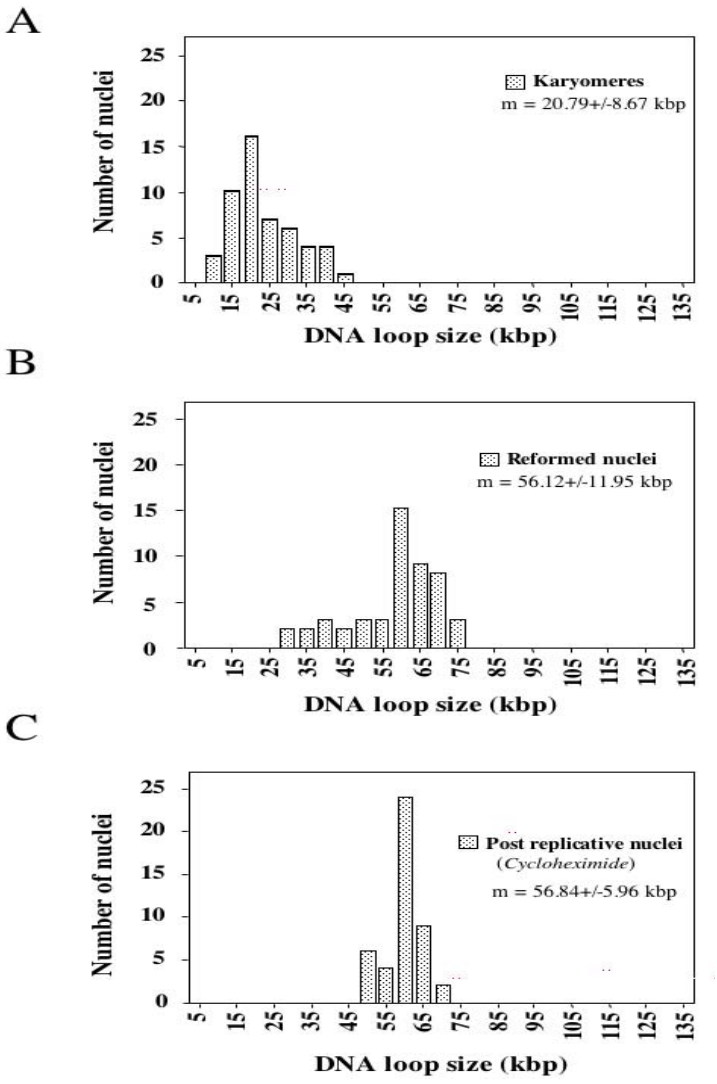

Figure S7. Loop-Size Increase within One Cell Cycle of 512-1024 Pre-MBT Embryos

Purified embryonic nuclei were submitted to loop size measurements as described in the Experimental Procedures. Since the cellular divisions are metasynchronous at this stage of development, we selected postmitotic nuclei (A) corresponding to early replicating karyomeres and reformed nuclei, and (B) corresponding to postreplicative nuclei (Lemaitre et al., 1998). We obtained similar results when comparing synchronized nuclei that had been prevented from entering mitosis using cycloheximide with reformed nuclei that corresponded to postreplicative nuclei (C).

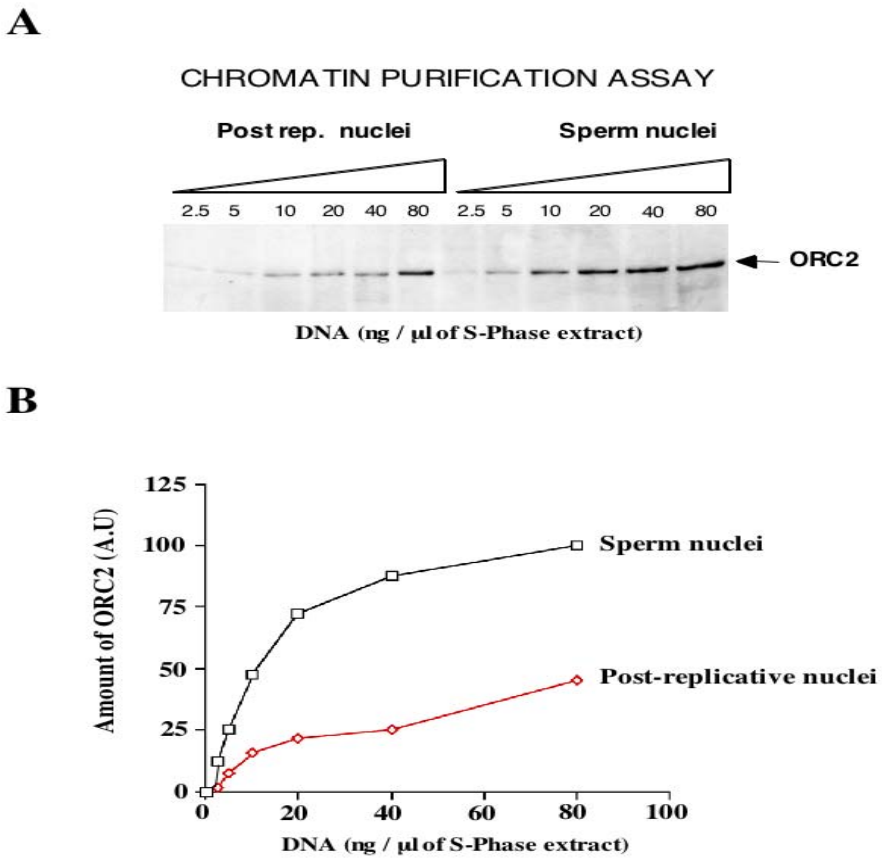

Figure S8. Replicon and ORC2 Binding in Sperm Nuclei or Postreplicative Embryonic Nuclei

(A) Demembranated sperm nuclei or demembranated postreplicative embryonic nuclei were incubated in S-phase extract. Chromatin was purified and proteins analyzed by SDS gel electrophoresis as described in Experimental Procedures.

(B) Quantitation from the immunoblot.