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Histone H1 modulates DNA replication through multiple pathways in *Xenopus* egg extract

Zhi Hong Lu, Donald B. Sittman, David T. Brown, Rajan Munshi and Gregory H. Leno*

Department of Biochemistry, University of Mississippi Medical Center, 2500 North State Street, Jackson, MS 39216-4505, USA
*Author for correspondence (e-mail: leno@fiona.umsmed.edu)

SUMMARY

We investigated the effects of histone H1s on DNA replication using *Xenopus* egg extract. Mouse variants H1c and $H1^0$ were assembled onto *Xenopus* sperm chromatin by the extract during the remodeling that accompanies nuclear decondensation. The association of H1 with chromatin was rapid and concentration dependent. H1-associated chromatin displayed a typical nucleosomal repeat pattern indicating that linker histones are properly positioned along the DNA. The presence of H1 on sperm chromatin reduced both the rate and extent of DNA replication in egg extract. This reduction in rate is due, in part, to a delay in initiation of replication within individual nuclei. Initiation in extract is dependent upon nuclear assembly. Analysis of the assembly process revealed that H1 does not inhibit nuclear membrane formation or the import of nuclear protein, however, it does slow the rate of nuclear lamina formation. This H1-induced delay in lamina assembly is responsible for the delay in initiation as pre-assembled H1-containing nuclei initiate replication at the same time as control nuclei. However, H1 inhibits replication even when lamina assembly is complete suggesting that H1 also affects replication directly. These data indicate that H1 modulates DNA replication through multiple pathways in egg extract.

Key words: DNA replication, Histone H1 variant, Nuclear assembly, Nuclear lamina, *Xenopus* egg extract

INTRODUCTION

Major transitions in chromatin structure and function occur during development in *Xenopus laevis*. These transitions are accompanied by programmed changes in individual chromosomal proteins (Newport and Kirschner, 1982; Dimitrov et al., 1993). Individual linker histone variants, which serve as important structural components and organizers of chromatin, are one example (reviewed by Khochbin and Wolffe, 1994). In early embryonic chromatin, the predominant linker histone is the cleavage stage variant B4 (H1M) (Smith et al., 1988; Hock et al., 1993; Dimitrov et al., 1993; Dworkin-Rastl et al., 1994). However, by the mid blastula transition (MBT), somatic-type linker histones, such as H1A, progressively accumulate and by the end of gastrulation have completely replaced B4 within embryonic chromatin (Dimitrov et al., 1993; Hock et al., 1993; Dworkin-Rastl et al., 1994). This transition is temporally correlated with the appearance of prolonged and asynchronous cell cycles (Newport and Kirschner, 1982). By stage 45, the differentiation-specific variant, $H1^0$, accumulates when the majority of cells are in the G_0/G_1 phase of the cell cycle (Grunwald et al., 1995).

Differential expression of linker histone variant genes during development may have important functional significance. Recent in vitro reconstitution studies have shown that both protein B4 and another chromosomal protein, HMG1, can bind specifically to linker DNA in a similar way as histone H1, although with lower affinity (Nightingale et al., 1996; Ura et al., 1996). B4 and HMG1 are present in *Xenopus* early embryonic chromatin at approximately one molecule for every two nucleosomes (Dimitrov et al., 1994) and it has been suggested that weak interaction of these proteins with linker DNA and core histones may induce the formation of less stable and more extended cleavage stage chromatin. These interactions may facilitate the exceptionally rapid early embryonic cell cycles when frequent reorganization of chromatin is essential (Nightingale et al., 1996; Ura et al., 1996). This view is consistent with the fact that replacement of B4 and HMG1 with the somatic-type linker histone, H1, is temporally correlated with the appearance of elongated and asynchronous cell cycles (Newport and Kirschner, 1982) and the formation of more compact embryonic chromatin at the MBT (Gurdon, 1968). By stage 45, there is a dramatic reduction in the number of proliferating cells within the embryo, and this reduction is correlated with the accumulation of the differentiation-specific linker histone, $H1^0$ (Grunwald et al., 1995).

There is clear evidence that the replacement of B4 and HMG1 with somatic H1 leads to dominant and specific repression of oocyte 5S rRNA gene transcription in vivo (Bouvet et al., 1994; Kandolf, 1994). This accumulation of H1 reduces accessibility of transacting factors to the oocyte genes (Chipev and Wolffe, 1992) possibly through chromatin compaction and stabilization of higher-order structure (Wolffe, 1989) or by a reduction in nucleosome mobility (Ura et al., 1995). Moreover, replacement of the somatic linker histones, H1 and $H1^0$, with the cleavage-stage linker protein B4 and HMG1 results in the acquisition of transcriptional competence in nuclei from terminally differentiated cells (Dimitrov and Wolffe, 1996). Col-

lectively, these studies support the view that somatic H1 can repress transcriptional activity both in vivo and in vitro; however, to what extent modulation of this activity by H1 affects the embryonic cell cycle during development is not clear. It is possible that somatic H1 variants also affect other important processes in the embryonic cell cycle such as DNA replication, and that these effects alter the timing and duration of the embryonic cell cycle during development.

The question of whether or not individual H1 variants play specific roles in regulating the embryonic cell cycle in *Xenopus laevis* has not been addressed experimentally. However, evidence supporting a regulatory role for H1 variants in cell cycle progression has been reported in studies with avian and mammalian somatic cells (Bergman et al., 1988; Sun et al., 1989; Aubert et al., 1991; Brown et al., 1996). For example, overexpression or microinjection of the differentiation-specific linker histone, H5, in proliferating somatic cells leads to a transient inhibition of DNA replication and arrest of cell proliferation (Sun et al., 1989; Berman et al., 1988; Aubert et al., 1991). Furthermore, differential effects of H1 variant overexpression on cell cycle progression were recently demonstrated in mammalian cells by Brown et al. (1996) who found that overexpression of the apparent histone H5 homologue in mammals, $H1^0$, results in a transient inhibition of both G_1 and S phase progression in mouse fibroblasts. H1c had no inhibitory effect on cell cycle progression. Although transcriptional effects are implicated in the inhibition of cell cycle progression by these H1 variants, the molecular mechanism(s) responsible for these effects are not known (Sun et al., 1989; Brown et al., 1996).

Extracts derived from *Xenopus laevis* eggs are capable of carrying out many of the cellular activities that occur in early embryos such as rapid chromatin assembly (Laskey et al., 1977), pronuclear formation (Lohka and Masui, 1983, 1984), DNA replication (Lohka and Masui, 1983; Blow and Laskey, 1986, 1988) and mitotic entry and exit (Murray and Kirschner, 1989). It is now clear that nuclear structure plays a major role in regulating DNA replication in this system (reviewed by Laskey and Madine, 1996) and therefore, egg extracts can be used to study the effects of individual linker histones on DNA replication as well as those processes that lead to the establishment of replication competence, namely chromatin and nuclear assembly. Such studies should provide insights into the role(s) H1 plays in establishing higher order chromatin structure and the developmental regulation of DNA replication.

In this report, we have investigated the effects of H1 variants on nuclear assembly and DNA replication in *Xenopus* egg extract. *Xenopus* sperm nuclei were incubated in egg extract supplemented with the H1 variants, H1c and $H1^0$, that were purified from H1 variant overexpressing mouse fibroblasts (Brown et al., 1996). H1 was assembled onto sperm chromatin by the extract during the remodeling that normally accompanies nuclear decondensation. Micrococcal nuclease digestion revealed that H1-associated chromatin displays a typical nucleosomal repeat pattern indicating that linker histones are properly positioned along the DNA. The presence of H1 on sperm chromatin significantly reduced both the rate and extent of DNA replication in egg extract. This reduction in rate is due, at least in part, to a delay in the initiation of replication within individual nuclei. An analysis of nuclear assembly revealed that H1 does not inhibit nuclear membrane formation or the import of nuclear protein, however, it does slow the rate of nuclear lamina formation. This H1-induced delay in lamina assembly is responsible for the delay in replication initiation as pre-assembled H1-containing nuclei initiate replication as efficiently as control nuclei. However, the extent to which these H1-containing nuclei replicate is still dramatically reduced relative to control nuclei. These data indicate that H1 modulates DNA replication through multiple pathways in egg extract. Variant-specific differences were not observed regarding the effects of H1 on nuclear lamina assembly or DNA replication, however, variant-specific differences in apparent nucleosome repeat length were observed.

MATERIALS AND METHODS

Purification of histone H1

The histones H1c and $H1^0$ were purified from their respective H1 variant overexpressing mouse cell lines according to the method of Brown et al. (1996) with the following modifications. The H1-containing HPLC fractions were pooled and dried by vacuum centrifugation. Dried samples were dissolved in deionized water prior to use. [^{35}S]Met-labeled H1 histones were prepared as described by Brown and Sittman (1993). Purity of isolated H1c and $H1^0$ was verified by SDS-polyacrylamide gel electrophoresis. A molar extinction coefficient of 17.84 M^{-1} cm^{-1} at 210 nm was used to determine the protein concentration of each sample.

Remodeling and decondensation of sperm nuclei

Egg extracts were prepared from activated eggs of *Xenopus laevis* essentially as described by Leng and Leno (1997) but without the addition of EGTA. Demembranated *Xenopus* sperm nuclei were prepared as described (Leng and Leno, 1997) and incubated at 77 ng DNA per microliter egg extract which was supplemented with an energy regenerating system (150 μg/ml creatine phosphokinase, 60 mM phosphocreatine), 100 μg/ml cycloheximide, 2 mM ATP, and histone H1 variants or an equivalent volume of water. Incubations were performed at 22°C for the times indicated for each experiment. The extent of extract dilution was kept constant at 30% for all chromatin remodeling and decondensation experiments.

Electrophoretic analysis of chromatin-bound proteins

Remodeled sperm chromatin was diluted 70-fold with buffer A (15 mM Tris-HCl, pH 7.4, 60 mM KCl, 15 mM NaCl, 1 mM β-mercaptoethanol, 0.5 mM spermidine, 0.15 mM spermine) and pelleted by centrifugation at 1,450 ***g*** for 10 minutes at 4°C in a Juan CR4-22 centrifuge. The chromatin pellet was washed once in buffer A, and repelleted by centrifugation at 1,450 ***g*** for 10 minutes at 4°C. Basic proteins were extracted from the isolated chromatin by addition of HCl to a final concentration of 0.5 M. Samples were spun in a bench top centrifuge for 10 minutes at 4°C and the resultant supernatant was frozen in liquid nitrogen and lyophilized. The proteins were resolved on 17% SDS-polyacrylamide gels using the Laemmli buffer system (Laemmli, 1970) and visualized using a Silver Stain Plus kit (Bio-Rad). For experiments involving [^{35}S]Met-labeled H1 variants, the polyacrylamide gels were dried and radioactivity was quantitated using a phosphorimager (Molecular Dynamics). The ratio of chromatin-bound radioactivity to total input radioactivity was used to calculate the H1/nucleosome (220 bp of DNA) ratio of H1-containing chromatin. Analysis of chromatin-associated proteins by Triton-acid-urea (TAU)/SDS 2-dimensional (2-D) gel electrophoresis was carried out as previously described (Leno et al., 1996).

Micrococcal nuclease digestion of chromatin

Micrococcal nuclease (MN) digestion of chromatin was performed

according to the method described by Philpott and Leno (1992) with minor modifications. Sperm nuclei at 77 ng DNA/μl were incubated in egg extract without or with varying amounts of either H1c or $H1^0$ for 3 minutes. The remodeled chromatin was then diluted 10-fold with buffer A and centrifuged onto a cushion of 90% Percoll in buffer A (Sigma). Chromatin (5 μg DNA) was resuspended in 200 μl MN digestion buffer (15 mM Tris-HCl, pH 8.5, 60 mM KCl, 15 mM NaCl, 0.34 M sucrose, 15 mM β-mercaptoethanol, 0.5 mM spermidine, 0.15 mM spermine, 2 mM $CaCl_2$) and incubated with 0.05 U of micrococcal nuclease (Sigma) at 22°C for 3 minutes. 200 μl of MN termination buffer (20 mM Tris-HCl, pH 8.0, 20 mM EDTA, 0.5% SDS) was added along with 0.5 mg/ml proteinase K (Sigma) followed by incubation for 16 hours at 37°C. DNA was then purified by phenol-chloroform extraction followed by ethanol precipitation. Dried samples were dissolved in TE buffer containing 1 μg/μl RNase A and incubated at 37°C for 1 hour. DNA fragments were resolved on a 1.8% Metaphor agarose gel (FMC) with 1× TBE buffer in an 8 volt/cm electric field. Nucleosome repeat length was determined by estimating the size of DNA fragments on the basis of electrophoretic mobility. DNA molecular mass markers at 100 bp intervals were used to plot the standard electrophoretic mobility vs DNA fragment size interpolation curve. For each individual digestion, the sizes of 5 sequential fragments corresponding to nucleosome monomer through pentamer were determined and normalized to the number of nucleosomes. The nucleosome repeat length is the average of these five values.

In vitro nuclear assembly and DNA replication

Sperm nuclei at 77 ng/μl were incubated in egg extract without or with 5.68 μM H1c or $H1^0$ for 3 minutes at 22°C. Remodeled sperm chromatin was then diluted to 1 ng DNA/μl with fresh egg extract supplemented with 100 μCi/ml [α-^{32}P]dATP (NEN) or 20 μM 5-biotin-16-deoxyuridine triphosphate (biotin-16-dUTP; Boehringer Mannheim) and 5.68 μM H1c or $H1^0$ when appropriate. The extent of extract dilution was kept constant at 20% for all nuclear assembly and DNA replication experiments. dNTPs were added to a final concentration of 50 μM to re-adjust pool sizes following dilution. Incorporation of [α-^{32}P]dATP was determined as previously described (Leno and Munshi, 1994). DNA replication was expressed as a percentage of the control sample at the final time point.

To determine H1 specificity, sperm nuclei at 77 ng/μl were incubated in egg extract with 157 μg/ml H1c or H2A/H2B for 3 minutes. The H2A/H2B was purified from bovine kidney as previously described (Philpott and Leno, 1992). Remodeled sperm chromatin was then diluted to 1 ng DNA/μl with fresh egg extract supplemented with 100 μCi/ml [α-^{32}P]dATP (NEN) and 157 μg/ml H1c or H2A/H2B when appropriate. The extent of extract dilution was kept constant at 30% for all experiments. dNTPs were added to a final concentration of 50 μM to re-adjust pool sizes following dilution. Incorporation of [α-^{32}P]dATP was determined as described above and DNA replication was expressed as a percentage of the control sample at the final time point.

To assemble nuclei without initiation of replication, 400 to 500 nM microcystin-LR was added to egg extract without or with 5.68 μM H1c. Sperm nuclei were then added at 77 ng/μl and incubated in egg extract for 3 minutes at 22°C. Remodeled sperm chromatin was then diluted to 1 ng DNA/μl with fresh egg extract supplemented with 20 μM 5-biotin-16-deoxyuridine triphosphate (biotin-16-dUTP; Boehringer Mannheim), with or without 5.68 μM H1c and 400 to 500 nM microcystin-LR. After a 3 hour incubation, an aliquot of nuclei from each sample was removed and stained with anti-lamin L_{III} antibodies to confirm lamina assembly and fluorescent streptavidin to detect incorporated biotin dUTP. The remaining portion of each sample was then diluted with 990 μl extraction buffer (Blow and Laskey, 1986) and transferred to fresh extract containing 100 μCi/ml [α-^{32}P]dATP (NEN) or 20 μM 5-biotin-16-deoxyuridine triphosphate (biotin-16-dUTP; Boehringer Mannheim) with or without H1c and incubated for various times as indicated in Fig. 8.

Microscopy

Decondensation of sperm chromatin was monitored by staining unfixed nuclei in egg extract with Hoechst 33258 (5 μg/ml). Nuclear membranes were visualized by staining unfixed, extract-assembled nuclei with the lipid dye, Nile Red (0.15 μg/ml) and total DNA was visualized with Hoechst 33258. Nile Red fluorescence was viewed and photographed with an Odyssey laser confocal microscope (Noran Instruments). Microscopic detection of biotinylated dUTP incorporation was performed as previously described (Leng and Leno, 1997).

T7 RNA polymerase with or without the SV40 large T-antigen nuclear localization sequence (NLS) was expressed in *Escherichia coli* BL21 (Grodberg and Dunn, 1988) and purified according to the procedure described by Zawadzki and Gross (1991). Protein concentrations were determined using the DC protein assay kit (Bio-Rad). Nuclear protein import was assessed by incubating sperm chromatin at 77 ng/μl in egg extract with or without 5.68 μM H1c. After 3 minutes in extract, remodeled sperm chromatin was diluted to 1 ng DNA/μl with fresh extract and incubated for an additional 27 minutes to allow for nuclear membrane assembly. The final reaction mixture was 12 μl. 500 ng T7 RNA polymerase, either with or without an NLS, was added to control and H1c-containing samples which were then incubated an additional 30 minutes giving a total time in extract of 60 minutes. Samples were diluted, centrifuged onto coverslips, and processed as previously described (Leno and Munshi, 1997).

To assay nuclear lamina assembly around sperm chromatin remodeled in the presence or absence of H1, nuclear assembly and replication reactions (see above) were diluted 70-fold with buffer A, spun onto coverslip at 770 ***g*** at 4°C in a Juan CR4-22 centrifuge, and fixed by incubation for 15 minutes in methanol at –20°C. The nuclei were washed in buffer A, TTBS-BSA (0.1% Tween-20, 25 mM Tris-HCl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1.5% BSA), stained for 1 hour with mouse anti-lamin L_{III} monoclonal antibody, $L_0$46F7 (Lourim et al., 1996), diluted 1:250 in TTBS-BSA, and washed and stained with Texas Red-conjugated sheep anti-mouse secondary antibody for 1 hour. DNA was stained with Hoechst 33258.

RESULTS

The association of histone H1 with sperm chromatin is rapid and concentration dependent

The purpose of this study was two-fold. First, to determine the effects of somatic histone H1 on the early embryonic cell cycle, in particular, the process of DNA replication, and second, to determine the extent to which the differential effects of H1c and $H1^0$ on cell cycle progression, as previously observed (Brown et al., 1996), were mediated by differential effects on DNA replication. As the first step in addressing these questions, it was necessary to determine if exogenous H1 could be assembled on *Xenopus* sperm chromatin by *Xenopus* egg extract. Previous studies have shown that incubation of sperm nuclei in egg extract results in the decondensation and remodeling of the highly condensed chromatin. This remodeling is mediated by the acidic protein nucleoplasmin which removes sperm-specific basic proteins while assembling histones H2A and H2B onto the DNA, forming nucleosome cores (Laskey et al., 1977; Philpott et al., 1991; Philpott and Leno, 1992). The cleavage-stage linker histone B4 and HMG1 also associate with the decondensed chromatin (Dimitrov et al., 1994) possibly organizing linker DNA in the nucleosome (Nightingale et al., 1996).

Mouse somatic linker histone variants, H1c and $H1^0$, were used to study the kinetics of H1 association with sperm chromatin during remodeling in egg extract. Demembranated

sperm nuclei at 77 ng/μl were incubated in extract supplemented with 5.68 μM purified H1c or $H1^0$, or in control extract without exogenous H1, for various periods of time. The amount of H1 added to extract is comparable to the estimated amount of somatic linker histone present in a *Xenopus* embryo at the gastrula-neurula transition (GNT) stage, i.e. >45 ng/embryo (Dworkin-Rastl et al., 1994). At this concentration, the total H1/nucleosome ratio was estimated to be 10. After various times of incubation, chromatin was isolated and chromosomal proteins were extracted with acid, lyophilized, resolved on a SDS-polyacrylamide gel and visualized by silver staining (Fig. 1A). As previously reported, major changes in chromatin protein composition occurs within 3 minutes of incubation in the control extract (compare C/3 minute profile with profile of unincubated *Xenopus* sperm nuclei, 'XSN'; also see Philpott and Leno, 1992). Comparison of the basic protein profiles from control and H1c-containing nuclei reveals that chromatin remodeling occurs equally rapidly in the presence of histone H1 (compare C/3 minute profile with H1c/3 minute profile). Furthermore, analysis of the remodeled chromatin by Triton-acid-urea (TAU)/SDS 2-D gel electrophoresis demonstrated that the removal of sperm-specific basic proteins, X and Y, and the addition of core histones H2A and H2B to the chromatin occur at the physiological rate in the presence of H1c (data not shown). Most of the exogenous H1 that eventually associates with chromatin under these conditions does so within this initial 3 minute incubation period (open arrowhead). After 3 minutes, only a minor increase in associated H1 is observed (H1c/3 minutes-60 minutes). To quantitate the kinetics of association of H1 with chromatin, parallel experiments using [^{35}S]Met-labeled H1c were performed as described in Materials and Methods. As shown in Fig. 1B, the association of H1c with chromatin was biphasic, with 72% of the total bound H1 associating with sperm chromatin within the initial 3 minute remodeling period. These data demonstrate that H1 associates with chromatin at a rate comparable to that of the endogenous basic proteins B4 (closed arrowheads) and HMG1 (asterisk) (C/3 minutes) whose identity was confirmed by TAU/SDS 2-D gel electrophoresis (see below). The association of H1 observed at later times may reflect the gradual maturation of the nascent chromatin (Philpott and Leno, 1992).

The association of H1c with sperm chromatin is accompanied by a small reduction in two major proteins (Fig. 1A; compare C/3 minutes with H1c/3 minutes and Fig. 1C; compare 0/1 with 10/1, closed arrowheads and asterisk). By comparing Fig. 1A with TAU/SDS 2-D polyacrylamide gels of identical samples (data not shown) we were able to identify these two displaced proteins as the linker histone B4 (closed arrowheads) and HMG1 (asterisk) (Dimitrov et al., 1993, 1994; Dworkin-Rastl et al., 1994). These results are not too surprising given that both B4 and HMG1 specifically bind to linker DNA in a similar fashion as histone H1 (Ura et al., 1996) and therefore, competition among these proteins for linker DNA sites would be expected. However, the limited extent to which endogenous proteins are displaced by H1 suggests that other factors may regulate this process. The identity of the protein just below HMG1 (asterisk) in Fig. 1A (H1c) and 1C (10/1) is a modified form of H1c, which coelutes with H1c in reverse-phase HPLC (Brown and Sittman, 1993).

The association of H1 with sperm chromatin is concentration dependent. This is illustrated qualitatively in Fig. 1C which shows basic protein profiles from sperm chromatin

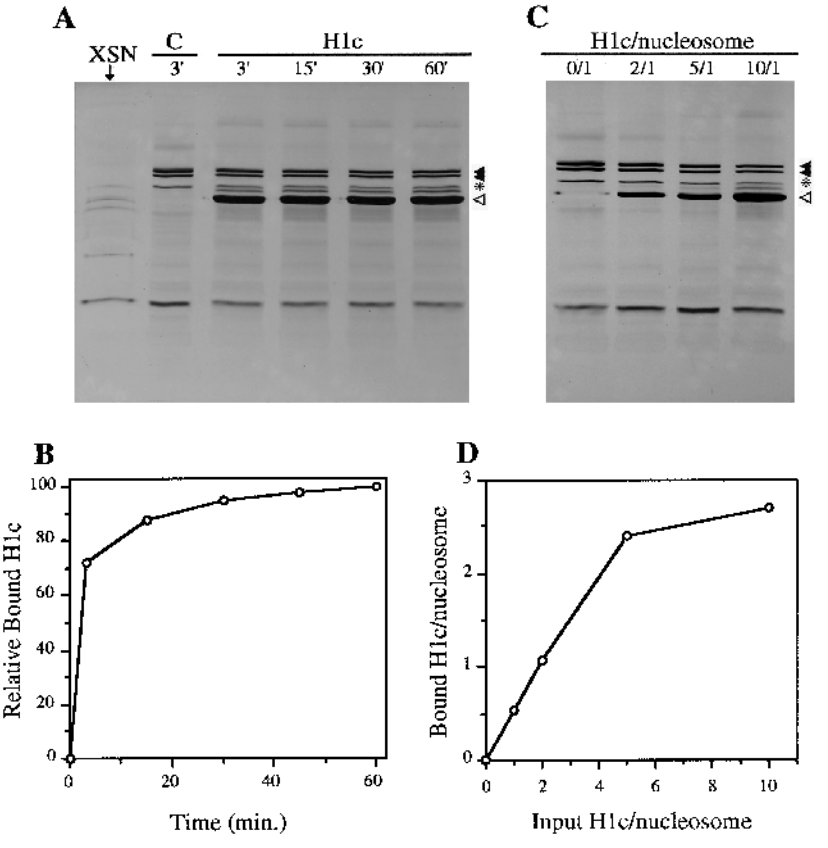


Fig. 1. Association of histone H1c with sperm chromatin is rapid and concentration dependent. (A) Permeable *Xenopus* sperm nuclei were incubated at 77 ng/μl extract, either lacking or containing 5.68 μM histone H1c. The reactions were incubated for 3 minutes in control extract lacking H1c (C) and for 3, 15, 30, and 60 minutes in H1-containing extract (H1c). Unincubated *Xenopus* sperm nuclei (XSN) and the remodeled chromatin samples were then pelleted, rinsed, and chromatin-associated proteins were extracted with acid, resolved by SDS-PAGE and visualized by silver-staining as described in Materials and Methods. (B) The same experiment as described in A was carried out except that [^{35}S]Met-labeled H1c was used. The kinetics of H1c association with chromatin is expressed as a percentage of the end-point chromatin-associated radioactivity. (C) Permeable sperm nuclei (77 ng/μl) were added to egg extract supplemented with H1c to the following concentrations: 0, 1.14, 2.84, 5.68 μM corresponding to H1/nucleosome ratios of 0, 2, 5, 10. The reactions were incubated for 3 minutes and processed as described in A. (D) The same experiment as described in C was carried out except that [^{35}S]Met-labeled H1c was used. Chromatin-associated radioactivity was used to assess the amount of H1c that was bound to chromatin. Association of H1c with chromatin is expressed as the ratio of bound H1c/nucleosome. (A and C) The positions of B4 (closed arrowheads), HMG1 (asterisk), and histone H1c (open arrowhead) are indicated.

remodeled in extract for 3 minutes following addition of varying amounts of exogenous H1c. The same experiment was also carried out with [^{35}S]Met-labeled H1c and the results quantitated with a phosphorimager (Fig. 1D). The association of H1c with chromatin was nearly linear up to an estimated input H1/nucleosome ratio of 5, an amount of H1 nearly equivalent to that found in the *Xenopus* embryo at the end of gastrulation, i.e. ~45 ng (Dworkin-Rastl et al., 1994). A higher concentration of H1c (5.68 μM) leads to only a minor increase in the ratio of bound H1/nucleosome. Thus, at an input H1 to nucleosome ratio of 10 (5.68 μM), the estimated ratio of bound H1c per nucleosome was actually less than 3. Results virtually identical to those described in Fig. 1 were obtained when H1^0 was used in place of H1c (data not shown).

Association of histone H1 promotes compaction of remodeled sperm chromatin

Histone H1 does not prevent the remodeling of sperm chromatin that normally accompanies decondensation in egg extract (Fig. 1). However, previous studies have shown that H1 can induce compaction of chromatin both in vivo and in vitro (Nelson et al., 1979; Allan et al., 1981; Affolter et al., 1987; Wolffe, 1989; Matsuoka et al., 1994; Halmer and Gruss, 1995). Therefore, we examined the effects of H1 on extract-mediated chromatin decondensation. Sperm nuclei were incubated in extract with or without 5.68 μM H1c and aliquots of remodeled chromatin were removed at various times for microscopic examination of total DNA (Fig. 2). Nuclei were photographed without extract incubation ('Sperm') or following 3, 15, and 30 minute incubations in H1c-free ('Control') or H1c supplemented extract ('H1c'). A clear difference in the extent of chromatin decondensation was observed between nuclei from control and H1c samples after only 3 minutes in the extract. Nuclei containing H1c (Fig. 1A, H1c/3 minutes) were consistently more compact than control nuclei. This is most evident when examining nuclear length. Even after 30 minutes in extract, nuclei containing H1c were more compact than control nuclei, however, in this case, a difference in nuclear width is most apparent. Taken together, these data demonstrate that histone H1 limits the extent of sperm decondensation in egg extract without disrupting the normal process of chromatin remodeling.

Histone H1 increases the nucleosome repeat length on sperm chromatin remodeled in egg extract

To determine if histone H1 alters the subunit structure of sperm chromatin following remodeling by egg extract, we incubated sperm nuclei for 3 minutes in extract either without H1 or with various amounts of H1c or H1^0. The chromatin was then isolated, digested with micrococcal nuclease and the endonuclease-resistant DNA fragments were resolved by agarose gel electrophoresis. A ladder of fragments, representing nucleosome monomers, dimers, trimers, tetramers, etc., were present in both control chromatin (lane 0/1; Fig. 3A and B) and H1-containing chromatin (lanes 2/1-10/1; Fig. 3A and B). However, in the presence of H1, the nucleosome repeat length increased relative to the control, and this increase was both concentration and variant dependent. A repeat length of 186 to 192 base pairs (bp) was observed in control chromatin (Fig. 3C), a range that is consistent with previous reports (Laskey et al., 1977; Almouzni and Mechali, 1988). In H1-containing chromatin, however, the nucleosome repeat length increased progressively with increasing H1 concentration. While this effect was observed with both H1 variants, the greatest change was observed with H1^0 (Fig. 3C). A reduced sensitivity to micrococcal nuclease digestion was also observed at high H1/nucleosome ratios, i.e. >5/1, the most dramatic change occurring with H1^0 (Fig. 3B, lane 4).

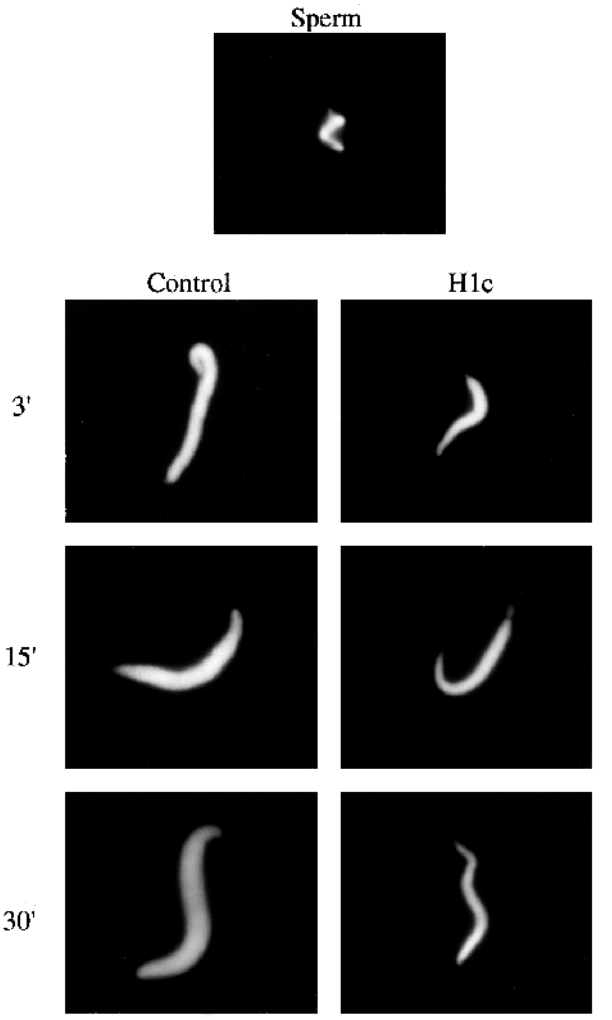
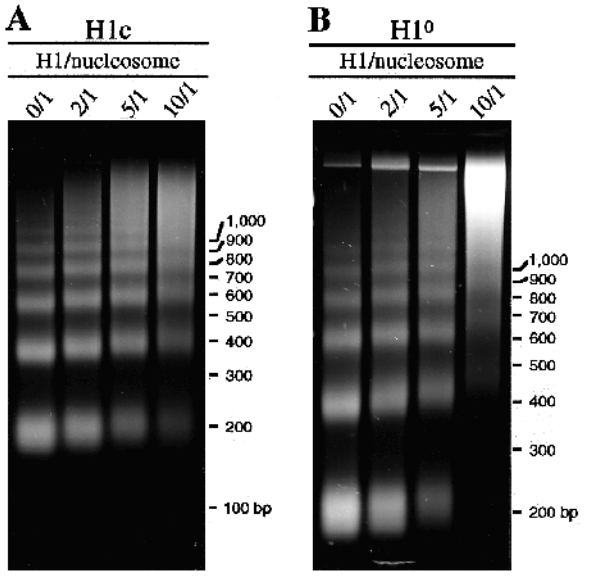


Fig. 2. Association of histone H1 induces compaction of sperm chromatin. Permeable sperm nuclei were added to egg extract either lacking (Control) or containing 5.68 μM H1c (H1c) corresponding to an H1/nucleosome ratio of 10. The reactions were incubated for 3, 15, and 30 minutes. Unincubated sperm nuclei (Sperm) or unfixed remodeled chromatin was stained with the DNA dye, Hoechst 33258 and viewed by fluorescence microscopy. The extent of decondensation is judged by changes in both the length and the width of chromatin.

Histone H1 inhibits DNA replication in *Xenopus* egg extract

Exogenous histone H1 associates with sperm chromatin during remodeling (Fig. 1) and modulates nucleosome repeat length in a concentration and variant dependent manner (Fig. 3). Collectively, these results suggest that H1 is properly assembled on sperm chromatin by egg extract and argue that this H1-containing chromatin can be used to study the effects of somatic



C **Nucleosome Repeat Length**
(base pair)

H1 variant	H1/nucleosome			
	0/1	2/1	5/1	10/1
H1c	186	192	201	212
$H1^0$	192	203	211	230

Fig. 3. Histone H1 preserves normal nucleosome structure but increases nucleosome repeat length in remodeled sperm chromatin. Permeable sperm nuclei were incubated at 77 ng DNA/μl egg extract containing H1c (A) or $H1^0$ (B) to the following concentrations: 0, 1.14, 2.84, 5.68 μM corresponding to H1/nucleosome ratios of 0, 2, 5, 10. After 3 minutes, samples were diluted and chromatin was isolated by centrifugation and digested with micrococcal nuclease at room temperature for 3 minutes. The DNA was purified, resolved on a 1.8% metaphor agarose gel with 1× TBE buffer as described in Materials and Methods. Molecular mass markers at 100 bp intervals are shown. (C) Nucleosome repeat lengths for control (0/1) and H1-containing chromatin, at H1/nucleosome ratios of 2/1, 5/1 and 10/1, are shown. In each experiment, the sizes of five sequential DNA fragments, corresponding to nucleosome monomer through pentamer, were determined on the basis of electrophoretic mobility and subsequently normalized to the number of nucleosomes. The average of these five values is considered the nucleosome repeat length for each sample.

H1s on DNA replication in this system. However, the conditions used to assemble H1 on sperm chromatin are not favorable for replication studies due to the high concentration of DNA present in the extract. To circumvent this problem, we diluted the remodeled chromatin with fresh egg extract prior to incubation, thus reducing the DNA concentration to a level that was suitable for replication assays. We also found that it was necessary to supplement the fresh extract with H1 in order to prevent its rapid loss from the remodeled chromatin upon dilution. This displacement of assembled H1 may be facilitated by the molecular chaperone, nucleoplasmin, which selectively removes H1 and $H1^0$ from *Xenopus* erythrocyte chromatin (Dimitrov and Wolffe, 1996).

To study the effects of H1 variants on the kinetics of DNA replication in egg extract we carried out time course experiments in which nascent DNA was labeled with [α-^{32}P]dATP (Fig. 4A). Specifically, sperm chromatin was added to egg extract and remodeled either in the absence of exogenous H1 (Control), or in the presence of purified H1c or $H1^0$ at a final concentration of 5.68 μM. After 3 minutes, the remodeled chromatin was diluted with fresh extract either without (Control) or with additional H1c or $H1^0$. In order to retain the assembled H1 on the chromatin after extract dilution, it was necessary to add H1 to the extract to a final concentration of 5.68 μM. The samples were then incubated for various times and processed as described in Materials and Methods. Fig. 4A shows the mean values along with the standard error of the mean from three separate experiments in which three different extracts were used. DNA replication is expressed as a percentage of the control sample at 210 minutes. Following a 30 minute delay, during which nuclear assembly occurs, incorporation of label in the control sample increased rapidly reaching a plateau by 90 to 150 minutes. The mass of DNA synthesized during this period is approximately equal to the mass of input DNA, i.e. ~1.0 ng/μl extract, demonstrating that virtually all nuclei underwent complete replication in the extract. In contrast, the presence of H1 reduced both the rate and the extent of replication by the extract. Thus, by 90 minutes, the time required to replicate nearly all of the DNA in the control sample, only ~30 % of the input DNA was replicated in the H1c and $H1^0$ samples.

Sperm nuclei act as individual and integrated units of DNA replication in egg extract (Blow and Watson, 1987). Thus, H1 could inhibit DNA replication in this system by delaying the timing of initiation within individual nuclei. To determine if the timing of initiation is affected by H1, we carried out time course studies in which nascent DNA was labeled with biotinylated dUTP and visualized by staining with fluorochrome-conjugated streptavidin (Blow and Watson, 1987). Specifically, nuclei were incubated in egg extract as described for Fig. 4A except that the extract was supplemented with biotinylated dUTP instead of [^{32}P]dATP. Incubated nuclei were isolated at 30, 60 and 180 minutes, fixed, stained with Hoechst 33258 to label total DNA, and with fluorescein-streptavidin to detect incorporated biotin-dUTP. The percentages of biotin-labeled nuclei in each sample are shown in Fig. 4B. After 30 minutes in the extract, only 11% of the control nuclei had incorporated biotin (Fig. 4B, 30 minutes, C) consistent with the fact that initiation of replication occurs only after nuclear assembly is complete and that completion of the assembly process requires approximately 30 minutes in our extracts (data not shown, see Leng and Leno, 1997). However, after 60 minutes in extract, over 90% of control nuclei were labeled with biotin demonstrating that most nuclei initiate replication during the 30 minute period following completion of the assembly process (Fig. 4B, 60 minutes, C). In contrast, only 2% of the H1-containing nuclei were labeled with biotin at 30 minutes (Fig. 4B, 30 minutes, H1c and $H1^0$) and less than 60% were labeled after 60 minutes in extract (Fig. 4B, 60 minutes, H1c and $H1^0$). By 180 minutes, however, 99% of control nuclei (Fig. 4B, 180 minutes, C) and most (91% H1c and 89% $H1^0$) H1 nuclei were biotin-labeled (Fig. 4B, 180 minutes, H1c and $H1^0$). Taken together, these data demonstrate that histone H1 delays

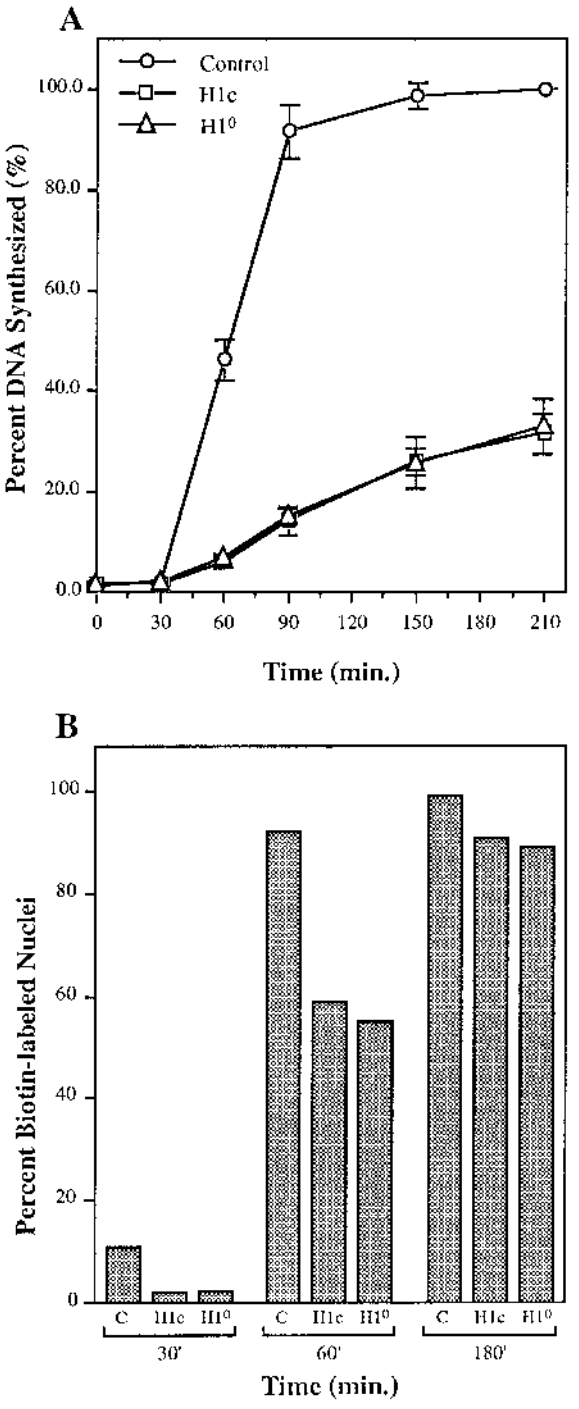


Fig. 4. Histone H1 inhibits DNA replication in egg extract. Permeable sperm nuclei were incubated at 77 ng DNA/μl extract either without (control, C) or with H1c (H1c) or $H1^0$ ($H1^0$) at an H1/nucleosome ratio of 10 for 3 minutes. The remodeled chromatin was then diluted to 1 ng DNA/μl in fresh extract containing [α-^{32}P]dATP (A) or 20 μM biotinylated dUTP (B) for various times as indicated. In the H1-containing samples, H1c or $H1^0$ were added to the fresh extract to a final concentration of 5.68 μM before dilution. The reactions were processed as described in Materials and Methods. (A) The mean values, along with the standard error of the mean, from three separate experiments in which three different extracts were used. DNA replication is expressed as a percentage of the control sample at 210 minutes. (B) Nuclei were stained with Hoechst 33258 to label total DNA and with Texas Red-streptavidin to detect biotin-dUTP incorporated into nascent DNA. Two hundred nuclei from each sample were examined for streptavidin fluorescence and the percent of biotin-labeled nuclei in each sample is shown.

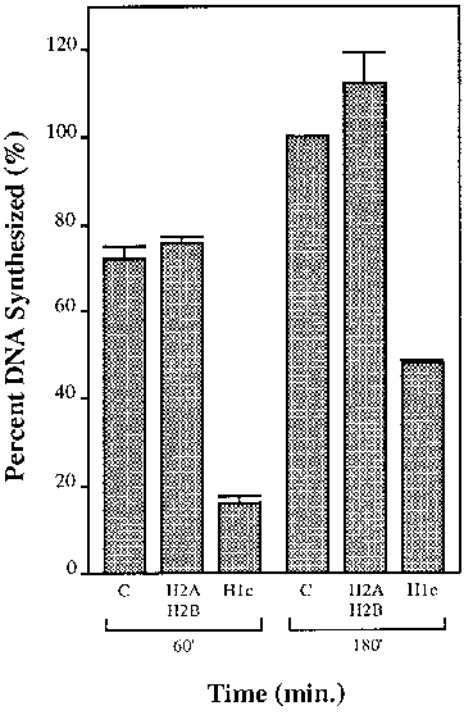


Fig. 5. Somatic core histones, H2A and H2B, do not inhibit DNA replication in egg extract. Permeable sperm nuclei were incubated at 77 ng DNA/μl in control extract (C) or extract containing 157 μg/ml H1c (H1c) or H2A/H2B (H2A/H2B) for 3 minutes. The remodeled chromatin was then diluted to 1 ng DNA/μl extract containing [α-^{32}P]dATP and exogenous histones as described in the remodeling reaction. Nuclei were incubated for various times as indicated. The reactions were processed as described in Fig. 4. DNA replication is expressed as percentage of the DNA synthesized in the control sample at 180 minutes.

the timing of initiation within individual nuclei in a variant independent manner.

To confirm that the inhibition of replication observed in Fig. 4A is specific for histone H1, we carried out virtually identical experiments in which the core histones, H2A and H2B, were added to egg extract in place of H1. All three histones have very similar isoelectric points and each interacts with the molecular chaperone, nucleoplasmin (Dilworth et al., 1987; Dimitrov and Wolffe, 1996), making these core histones a good control for H1 specificity. Fig. 5 shows the mean values along with the standard error of the mean from two experiments using two different egg extracts supplemented with 157 μg/ml of H2A/H2B or H1. DNA replication is expressed as a percentage of the control sample at 180 minutes. Unlike H1c, H2A and H2B had no inhibitory effect on DNA replication in the extract. Thus, histone H1 reduces replication in egg extract while core histones of nearly identical charge do not demonstrating that inhibition is specific for H1.

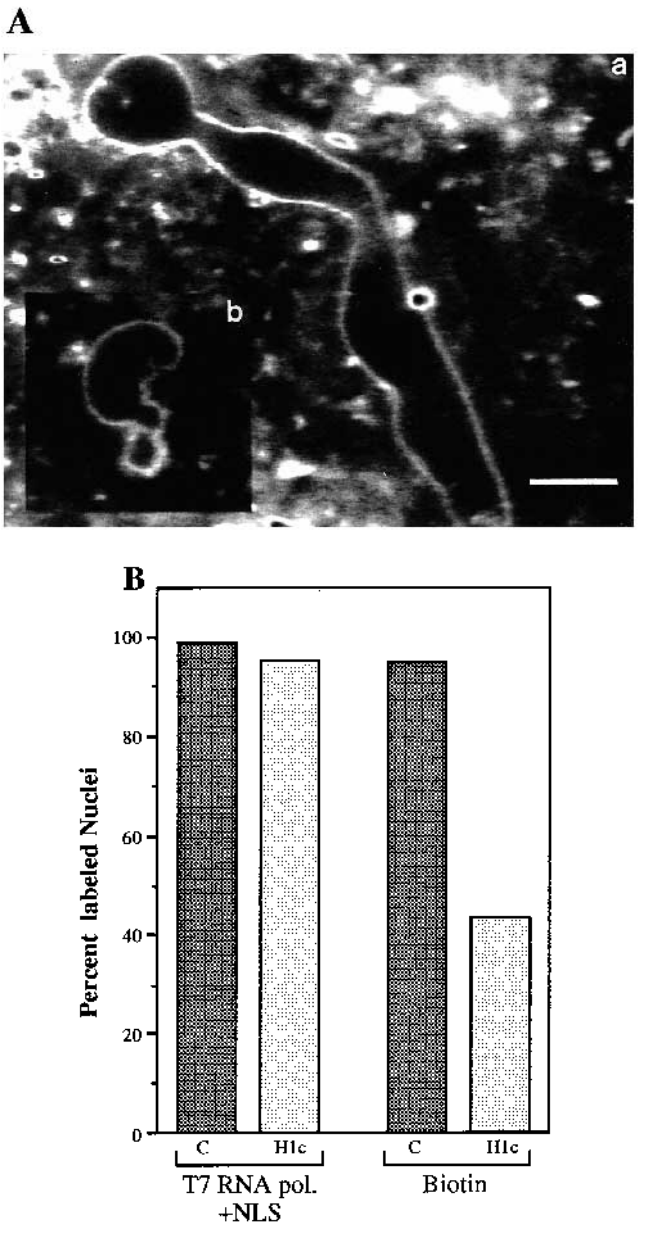


Fig. 6. H1 does not inhibit nuclear membrane assembly or nuclear protein import. (A) Permeable sperm nuclei were remodeled and diluted with extract either without or with H1c as described in Fig. 4. The reactions were incubated for 30 minutes. Unfixed nuclei were stained with Nile Red and viewed by confocal microscopy. A typical nucleus assembled in control extract without H1 (a) or extract with H1c (b, inset) is shown. A bright, continuous fluorescence was observed around virtually all sperm nuclei in both samples indicating the presence of complete nuclear membranes. Bar, 3 μm.
(B) Permeable sperm nuclei were remodeled for 3 minutes in extract without (control, C) or with H1c (5.68 μM) and then diluted in fresh extract supplemented with biotin-dUTP and H1c (5.68 μM) when appropriate. The samples were incubated for 27 minutes to allow nuclear membrane formation. T7 RNA polymerase tagged with the SV40 large T antigen nuclear localization sequence (NLS) was then added to all reactions and incubated for another 30 minutes. Nuclei were isolated, fixed and stained with rabbit anti-T7 polyclonal antiserum and with fluorescein-conjugated anti-rabbit antibody. The incorporated biotin-dUTP was detected with Texas Red-streptavidin. Two hundred nuclei from each sample were examined for both T7 and streptavidin fluorescence. The percentages of T7 RNA polymerase-labeled nuclei (T7 RNA pol. +NLS) and biotin-labeled nuclei (Biotin) are shown.

Histone H1 does not inhibit nuclear membrane formation or nuclear protein import

The nuclear membrane determines the timing of initiation of replication in egg extract (Leno and Laskey, 1991). Therefore, the H1-dependent delay in initiation described above could be due to disruption of nuclear membrane assembly. To test this hypothesis, we remodeled sperm nuclei in egg extract for 3 minutes, either in the absence (control) or presence of H1c (5.68 μM). Following remodeling, samples were diluted with unsupplemented extract (control) or extract containing H1 (5.68 μM) and incubated for an additional 27 minutes. Fresh, unfixed nuclei from each sample were then stained with the lipid dye, Nile Red and viewed by confocal microscopy (Fig. 6A). A continuous, peripheral Nile Red fluorescence was observed in virtually all control (a) and H1-containing nuclei (inset, b) after 30 minutes in the extract indicating complete nuclear membrane assembly around sperm chromatin (Cox and Leno, 1990; Leng and Leno, 1997). Thus, under these conditions, histone H1 does not delay the initiation of replication by preventing formation of a complete nuclear membrane.

H1 does, however, have a dramatic effect on nuclear size (Fig. 6A). Control nuclei (a) were consistently larger than H1-containing nuclei (inset, b) raising the possibility that H1 limits membrane-dependent nuclear swelling, and the initiation of replication, by disrupting nuclear protein import. To test this hypothesis, sperm nuclei were remodeled for 3 minutes without H1c (control) or with H1c (5.68 μM) as previously described. Each sample was then diluted with fresh extract containing biotin-dUTP with H1c (5.68 μM) or without H1c (control). The diluted samples were incubated in extract for an additional 27 minutes to allow nuclear membrane formation to occur. Purified T7 RNA polymerase, constructed with the nuclear localization signal (NLS) from the SV40 large T antigen (Benton et al., 1990), was then added to each sample and all samples were incubated an additional 30 minutes. Natural T7 RNA polymerase is too large to diffuse through the nuclear pore complex and, as such, is excluded from an intact nucleus. However, the polymerase containing the NLS is actively transported through the pore complex and accumulates within an intact nucleus (Dunn et al., 1988). Nuclei were isolated, fixed, and stained with Hoechst 33258, anti-T7 polymerase polyclonal antiserum and fluorescein-conjugated secondary antibody and Texas Red-streptavidin. Two hundred nuclei from each sample were examined for both polymerase and biotin fluorescence, and the percent labeled nuclei in each category is shown in Fig. 6B. Nearly all control nuclei accumulated T7 polymerase after the 60 minute extract incubation (99%) (Fig. 6B, T7 RNA pol. +NLS, C and H1c) and most of these transport competent nuclei also initiated replication (95%). In contrast, only 46% of the H1c nuclei replicated in a parallel incubation, even though most (96%) of these nuclei were capable of nuclear protein import. These data indicate that H1 does not delay initiation by inhibiting nuclear protein import within individual nuclei. However, we cannot rule out the possibility that H1 may have more subtle effects on nuclear

import and that these effects could contribute to the delay in initiation that we observe.

Histone H1 slows assembly of the nuclear lamina

The formation of a complete nuclear lamina by egg extract is a late event in the process of nuclear assembly (Hutchison et al., 1988; Hutchison and Kill, 1989). Thus, following formation of a transport-competent nuclear membrane, lamin L_{III}, the most abundant lamin species in egg extract, accumulates within the nucleus leading to the formation of a complete lamina structure and the initiation of DNA replication (Hutchison et al., 1994). However, if the majority of lamin L_{III} is removed from egg extract by immunodepletion, the resultant nuclei are incompetent for initiation (Newport et al., 1990; Meier et al., 1991; Jenkins et al., 1993). Thus, the initiation of DNA replication in egg extract requires the formation of a complete nuclear lamina. Therefore, we investigated the possibility that histone H1 delays the timing of initiation within individual nuclei by delaying the assembly of the nuclear lamina.

Sperm nuclei were remodeled in extract either without (control) or with H1c or $H1^0$ (bound H1/nucleosome ratio <3) for 3 minutes. Remodeled chromatin was then diluted to 1 ng DNA/μl with extract containing biotin dUTP and either H1c or $H1^0$ (5.68 μM) or without H1 (control). Samples were incubated for up to 180 minutes. At 30, 60 and 180 minute time points, the nuclei were isolated, fixed, stained with Hoechst 33258, the monoclonal antibody $L_0$46F7, which recognizes *Xenopus* lamin L_{III} (Lourim et al., 1996), and fluorochrome-conjugated streptavidin. Fig. 7A shows a single nucleus following incubation in the control extract for 180 minutes. Lamin L_{III} staining is clearly visible, lining the boundary of the nuclear membrane (Fig. 7A, compare panels c and a). Streptavidin fluorescence is also evident within this nucleus demonstrating the incorporation of biotin dUTP into nascent DNA (Fig. 7A, compare panels d and b). The results from a typical time course experiment are shown in Fig. 7B. Two hundred nuclei from each time point were classified as either lamin negative/biotin negative (L_{III}–B–, open bars), lamin positive/biotin negative (L_{III}+B–, stippled bars) or lamin positive/biotin positive (L_{III}+B+, filled bars). No nuclei showed biotin fluorescence alone, consistent with the notion that initiation of replication requires an intact nuclear lamina. Populations of control and H1-containing nuclei differed from one another in their rate of lamina assembly. Less than 59% of the H1-containing nuclei were lamin positive after 60 minutes in the extract as compared to over 80% of the control nuclei (Fig. 6B, 60 minutes, C, H1c and $H1^0$). Similar differences in the rate of DNA replication were also observed between control and H1-containing nuclei (Fig. 7B) consistent with the data shown in Fig. 4B. Although in the experiment described here, the actual rates of replication in both the control and H1 samples were somewhat lower than previously observed (Compare Fig. 7B with Fig. 4B). No variant-specific differences in the rate of lamin accumulation were observed between H1c- and $H1^0$-containing nuclei. Thus, histone H1 delays nuclear lamina assembly in egg extract.

The H1-induced delay in initiation is due to incomplete nuclear lamina assembly

The initiation of DNA replication in egg extract is dependent

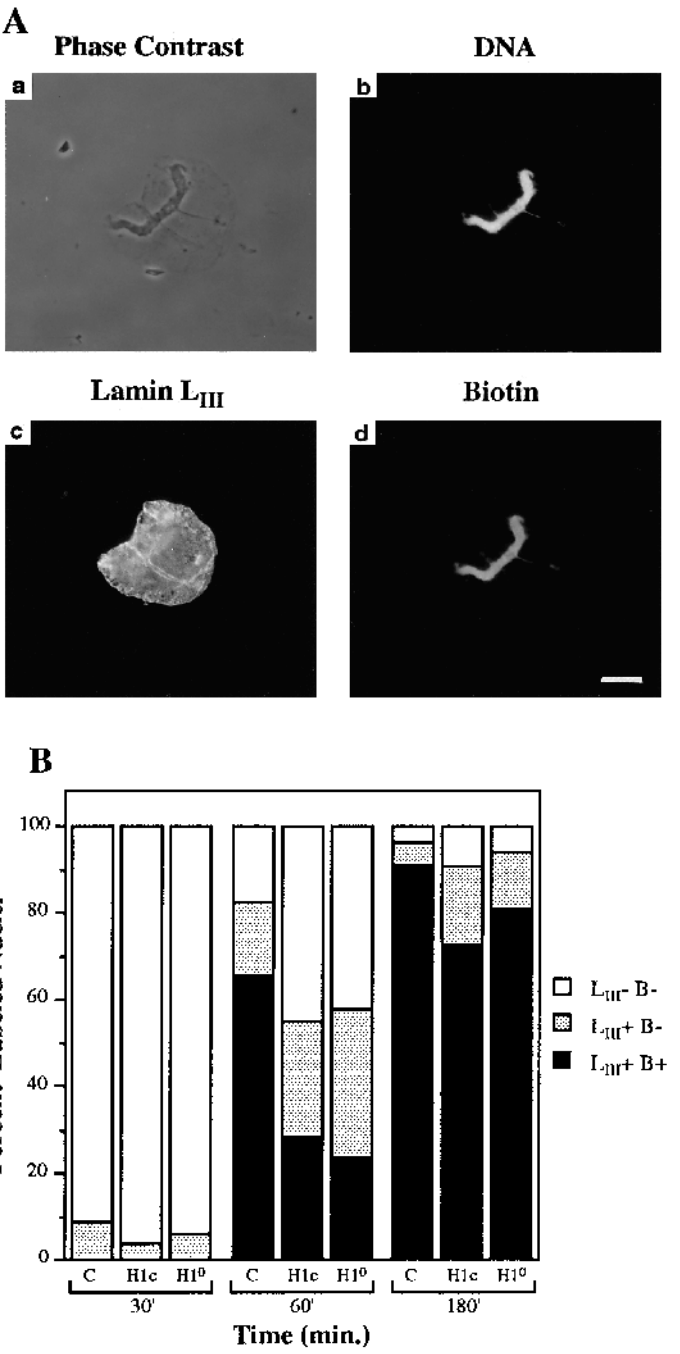


Fig. 7. Histone H1 slows assembly of the nuclear lamina. Permeable sperm nuclei were remodeled in extract without or with H1 variants to a final concentration of 5.68 μM for 3 minutes. Samples were then diluted to 1 ng DNA/μl extract supplemented with biotin dUTP and additional H1 (5.68 μM) when appropriate. Samples were incubated for various times as indicated. Nuclei were isolated and stained with mouse anti-lamin L_{III} monoclonal antibody, $L_0$46F7 and with Texas Red-conjugated anti-mouse secondary antibody. The incorporated biotin-dUTP was detected with fluorescein-streptavidin. (A) A single nucleus from the control extract incubated for 180 minutes. (a) Phase contrast, (b) DNA, (c) lamin L_{III}, (d) biotin. Bar, 5 μm. (B) Two hundred nuclei from each sample were examined for lamin and streptavidin fluorescence. Lamin L_{III} negative, biotin negative (L_{III}– B–); lamin L_{III} positive, biotin negative (L_{III}+ B–); lamin L_{III} positive, biotin positive (L_{III}+ B+).

upon the assembly of a nuclear lamina. Thus, the H1-induced delay in lamina assembly that we observe (Fig. 7) could alone account for the delay in the timing of initiation within individual nuclei (Figs 4 and 7). To test this hypothesis, we carried out experiments using the protein phosphatase inhibitor, microcystin. Microcystin reversibly inhibits the initiation of replication in egg extract without disrupting nuclear assembly (Murphy et al., 1995). Specifically, sperm nuclei were remodeled in extract supplemented with microcystin and either without (control) or with H1c (bound H1/nucleosome ratio <3) for 3 minutes. Remodeled chromatin was then diluted to 1 ng DNA/μl with extract containing biotin dUTP, microcystin, and either with H1c (5.68 μM) or without H1c (control). Samples were incubated for an additional 180 minutes, the time required for complete lamina assembly in the presence of H1 (Fig. 7B). Virtually all control and H1-containing nuclei possessed nuclear lamina after this 3 hour incubation; however, no initiation of replication was observed in nuclei from either sample (data not shown). Control and H1 samples were then diluted and the nuclei transferred to fresh extract without microcystin but containing biotin dUTP with or without H1. Aliquots were removed after 15, 30 and 90 minutes and the percent of biotin-labeled nuclei at each time point was determined (Fig. 8A). No significant difference in the number of biotin-labeled nuclei was observed between control and H1-containing samples at any time point examined demonstrating that H1 does not delay the initiation of replication once nuclear lamina assembly is complete. Thus, the H1-induced delay in initiation that we observe (Figs 4 and 7) is due, at least in part, to H1s affect on nuclear lamina assembly. However, a striking difference in the intensity of biotin fluorescence was observed between control and H1-containing nuclei in these experiments. Nearly all control nuclei showed bright streptavidin fluorescence at the 30 minute time point while fluorescence in H1-containing nuclei was significantly reduced relative to control nuclei even after 90 minutes in extract (data not shown). These data suggest that H1 inhibits replication even after nuclear lamina assembly is complete. To test this hypothesis, we carried out experiments essentially as described above except that [α-^{32}P]dATP was added to extract to label nascent DNA. Reactions were stopped after 15, 30, 45, 60 and 90 minutes in extract. Fig. 8B shows the mean values along with the standard error of the mean from three separate experiments in which three different extracts were used. DNA replication is expressed as a percentage of the control sample at 90 minutes. Clear differences in both the rate and extent of replication were observed between control and H1-containing nuclei. While >80% of H1-containing nuclei initiated replication after 15 minutes in the extract (Fig. 8A), <5% of the total DNA was replicated by this time (Fig 8B). By contrast, ~20% of the DNA was replicated in the control sample after 15 minutes in extract (Fig. 8B). Even after 90 minutes, <40% of the total DNA was replicated in the presence of H1 while replication was complete in control samples. Taken together, these data demonstrate that H1 modulates DNA replication through multiple pathways in egg extract.

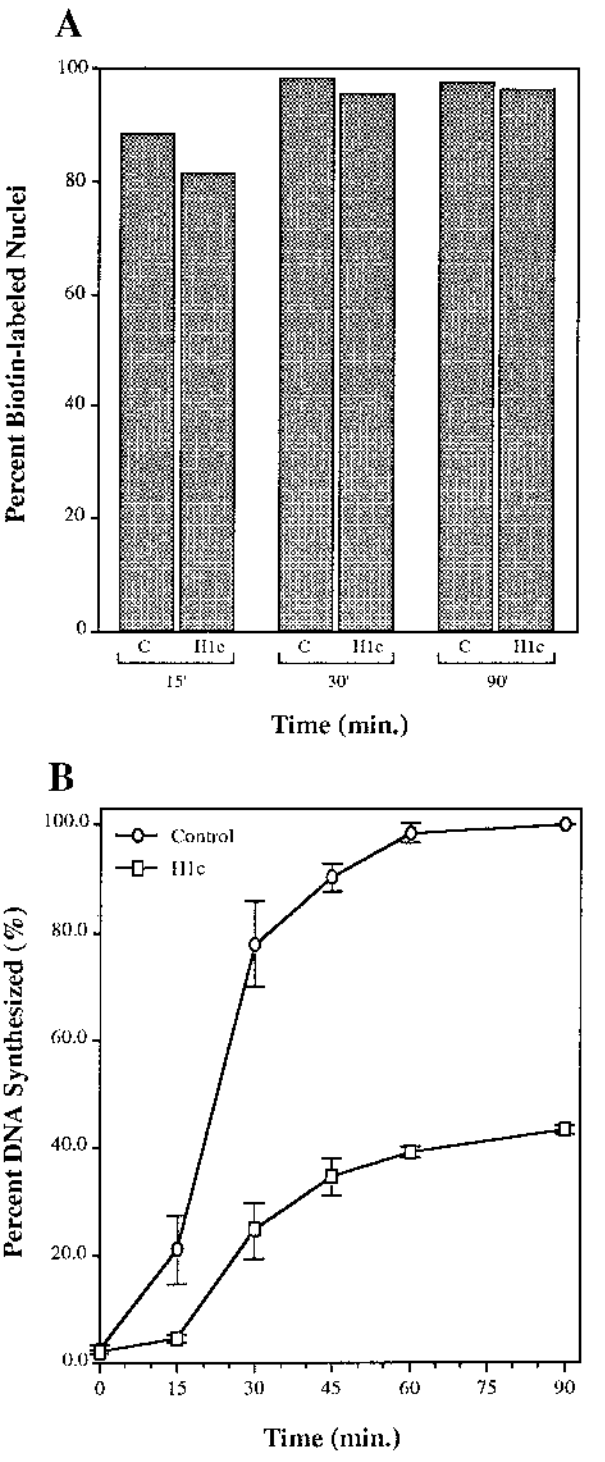


Fig. 8. Histone H1 inhibits DNA replication in pre-assembled nuclei. The protein phosphatase inhibitor, microcystin-LR, was added to egg extract at a final concentration of 400 to 500 nM. Permeable sperm nuclei were then added at 77 ng DNA/μl extract either without (Control, C) or with H1c (Hlc) at a final concentration of 5.68 μM. Samples were incubated for 3 minutes. The remodeled chromatin was then diluted to 1 ng DNA/μl with fresh microcystin-treated extract and incubated for 3 hours. The nuclei were then diluted with buffer and transferred to fresh extract containing 20 μM biotinylated dUTP (A) or [α-^{32}P]dATP (B) for various times as indicated.

DISCUSSION

The major conclusions of this work are four-fold. First, somatic

histone H1 can be assembled on sperm chromatin by egg extract. This assembly is rapid and concentration dependent (Fig. 1). H1-assembly promotes compaction of sperm chromatin (Fig. 2) and alters apparent nucleosome repeat length (Fig. 3). Second, histone H1 delays the timing of initiation of DNA replication within individual nuclei (Fig. 4). This delay in initiation is not due to disruption of nuclear membrane assembly (Fig. 6A) or nuclear protein import (Fig. 6B) but rather due to a delay in nuclear lamina assembly (Figs 7 and 8A). Third, H1 inhibits replication even when nuclear lamina assembly is complete (Fig. 8B) demonstrating that H1 also modulates replication by lamina-independent mechanisms. Fourth, variant-specific differences were not observed regarding the effects of H1 on nuclear lamina assembly or DNA replication, however, variant-specific differences in apparent nucleosome repeat length were observed (Fig. 3).

Effects of somatic H1 on chromatin structure

We have demonstrated that the somatic histone variants, H1c and $H1^0$, rapidly associate with sperm chromatin during remodeling by egg extract (Fig. 1 and data not shown). Our data indicate that H1 is properly assembled into chromatin. The evidence for this is twofold. First, assembly of H1 results in a specific displacement of the endogenous linker histone, B4, and HMG1 from chromatin without the loss of nucleosome cores (Fig. 1 and data not shown). It has recently been suggested that B4 and HMG1 play a role in the organization of *Xenopus* cleavage-stage chromatin by interacting with linker DNA (Dimitrov et al., 1994; Nightingale et al., 1996; Ura et al., 1996). Therefore, the displacement of B4 and HMG1 by H1 argues that the H1 is correctly positioned on the linker DNA in remodeled chromatin. However, not all B4 and HMG1 are displaced by H1, even when H1 is present in high concentration. This is somewhat surprising given that H1 has a higher affinity for linker DNA than either B4 or HMG1 (Ura et al., 1996). However, the proper positioning of linker histones on DNA during chromatin remodeling may involve extract-specific factors such as spacing factor (Tremethick and Frommer, 1992) or nucleoplasmin (Dimitrov and Wolffe, 1996) and these factors may alter the equilibrium of linker histone binding to DNA. Second, our micrococcal nuclease digestion studies indicate that H1 binding to chromatin does not disrupt normal nucleosome structure, i.e. the regular spacing of nucleosomes (Fig. 3). Interestingly, the nucleosome repeat length increased with increasing concentration of H1 and this change was variant-specific. In vitro reconstitution studies have provided evidence for a two-step model for the assembly of nucleosome structure in *Xenopus* oocyte and egg extracts. In this model, a nucleosome spacing activity, along with nucleosome cores, first establishes a primary nucleosomal array of about 165 bp repeat length. Addition of histone H1 then increases the repeat length to approximately 190 bp (Tremethick and Frommer, 1992). Proteins B4 and HMG1 may replace H1 function in oocyte and egg extracts, establishing a regularly spaced nucleosomal repeat length of approximately 180 bp (Dimitrov et al., 1994). Our results demonstrating that H1 variants alter nucleosomal spacing in egg extract are consistent with earlier work showing that the addition of somatic H1 to oocyte extract can increase the nucleosome repeat length of chromatin (Rodriguez-Campos et al., 1989; Shimamura et al., 1989).

There are a number of ways in which H1 could increase nucleosome repeat length. First, H1 may protect a more extended linker DNA than B4 and HMG1. This difference may be due to the fact that H1 has a higher affinity for linker DNA than the cleavage-specific linker proteins. Second, H1 may increase repeat length by restricting nucleosome sliding. During chromatin assembly, nucleosome cores are free to slide along the DNA until the binding of linker histone restricts their movement (Ura et al., 1995). H1 may be more efficient than B4 or HMG1 at restricting nucleosome movement by virtue of its greater binding affinity (Ura et al., 1996). Nucleosome repeat length has previously been shown to decrease during micrococcal nuclease digestion as a consequence of nucleosome sliding (Lohr et al., 1977). Thus, restricted sliding may lead to less trimming of H1-containing nucleosome arrays and generation of apparent longer repeat lengths (Lohr et al., 1977; Trieschmann et al., 1995). Third, an increase in repeat length could be due to H1-induced compaction of the chromatin. At higher H1 concentrations, an overall decrease in the amount of digested chromatin is observed (Fig. 3). Resistance of H1-containing chromatin to micrococcal nuclease could result in apparent longer repeat lengths due to limited trimming by the nuclease (Fig. 2) (Trieschmann et al., 1995).

Collectively, these data argue that the histone variants, H1c and $H1^0$, are properly assembled on sperm chromatin by egg extract and indicate that this H1-containing chromatin can be used to study the effects of somatic H1s on DNA replication in this system.

Effects of somatic H1 on DNA replication

We have shown that the somatic linker histone, H1, reduces both the rate and extent of DNA replication in egg extract (Fig. 4A). This effect is not variant specific however, as both H1c and $H1^0$ inhibit replication to the same extent. The reduction in replication rate is due, at least in part, to a delay in the initiation of replication within individual nuclei (Fig. 4B). Numerous studies have shown that initiation of replication in egg extract is dependent upon nuclear structure (reviewed by Laskey and Madine, 1996). For example, initiation requires an intact nuclear membrane (Newport, 1987; Sheehan et al., 1988; Blow and Sleeman, 1990; Leno et al., 1992; Coverley et al., 1993) that is capable of nuclear protein import (Cox, 1992). In addition, initiation of replication also requires the formation of a nuclear lamina, a late event in the assembly process (Newport et al., 1990; Meier et al., 1991; Jenkins et al., 1993). An analysis of the assembly process showed that H1 does not inhibit nuclear membrane formation or the import of nuclear protein (Fig. 6), however, it does slow the rate of nuclear lamina formation within individual nuclei (Fig. 7). This delay in lamina assembly is responsible for the delay in initiation as pre-assembled H1-containing nuclei initiate replication at the same time as control nuclei (Fig. 8A).

Assembly of a nuclear membrane, with functional pore complexes, precedes the formation of a nuclear lamina in *Xenopus* egg extract (Hutchison et al., 1988; Hutchison and Kill, 1989). Thus, the majority of lamin L_{III} enters the sperm nucleus by active transport through the nuclear pores and resides as a polymerized network of filaments underlying the inner nuclear membrane (Goldberg et al., 1995). In mammalian cell nuclei, lamins are also structural components of the nucleoskeleton (Hozak et al., 1995) and DNA replication

centers (Moir et al., 1994). However, in sperm nuclei, lamin L_{III} does not appear to be a component of the nuclear matrix, nor is a nuclear lamina required for matrix filament formation (Zhang et al., 1996). A nuclear lamina is necessary for matrix stabilization, however, and this stabilization appears to be required for initiation of replication by egg extract (Zhang et al., 1996). We have not investigated nuclear matrix assembly in our studies, but in light of the results of Zhang et al. (1996), it seems reasonable to speculate that the H1-induced delay in initiation that we observe may be due, in part, to the lack of a stable nuclear matrix.

The mechanism(s) by which histone H1 slows lamina assembly is not clear. One possibility is that H1 disrupts lamin-chromatin associations that are required for lamina assembly. This disruption could be due to global changes in chromatin structure, such as the H1-induced compaction of sperm chromatin that we observe (Fig. 2) or alternatively, to binding of H1 to specific regions of the DNA that are necessary for lamin interaction and lamina assembly. Interestingly, both histone H1 (Zhao et al., 1993) and lamins (Luderus et al., 1992) have been shown to interact with A/T rich scaffold or matrix attachment region DNA sequences. However, what role, if any, these interactions play in lamina assembly is not clear.

Histone H1 inhibits replication even when nuclear lamina assembly is complete (Figs 7B and 8) suggesting that H1 also modulates replication directly. This idea is supported by the observations of Halmer and Gruss (1995) who showed that H1 inhibits replication of reconstituted SV40 mini chromosomes in HeLa cell extract, a system that does not require nuclear lamina assembly for initiation (Halmer and Gruss, 1995). Moreover, we have found that H1 reduces the number of RP-A containing foci assembled on sperm chromatin by the extract (our unpublished observation). Assuming that RP-A foci are the precursors of replication foci (Adachi and Laemmli, 1992) and that each replication focus contains from 300 to 1,000 replication forks (Mills et al., 1989), it seems likely that even a small reduction in the number of RP-A foci would constitute a significant drop in the number of functional origins of replication. This raises the interesting possibility that H1 may limit replication by limiting origin use within each nucleus. Recently, however, it has been shown that RP-A foci can form efficiently on sperm chromatin depleted of Xorc2 or Xcdc6 (Coleman et al., 1996) suggesting that RP-A foci may not be the immediate precursors of replication foci as first thought. Therefore, we are currently investigating the effects of H1 on the binding of known pre-initiation complex proteins to chromatin.

A differential effect on cell cycle progression has been observed in mouse cells overexpressing $H1^0$ and H1c (Brown et al., 1996). $H1^0$ delayed cell cycle progression, but H1c did not. This difference was explained, in part, by a differential effect of these two variants on gene expression. However, differential effects on DNA replication could also contribute to this cell cycle delay. In somatic cells, S phase entry is dependent upon transcription and therefore, a direct effect of H1 variants on replication is difficult to demonstrate. In egg extract, however, replication occurs in the absence of transcription making this system useful for determining the effects of somatic H1 variants on replication apart from their effects on transcription. We found that both H1 variants inhibit DNA replication equally in egg extract (Fig. 4). This result was somewhat surprising given that H1c does not delay cell cycle progression in vivo (Brown et al., 1996). However, these differential effects on replication may simply relate to fundamental differences between embryonic and somatic cell cycles. Thus, in egg extract, H1c may affect both nuclear assembly and DNA replication creating a more extended S-phase, similar to S-phase in somatic cells.

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REFERENCES

Adachi, Y. and Laemmli, U. K. (1992). Identification of nuclear pre-replication centers poised for DNA synthesis in *Xenopus* egg extracts: Immunolocalization study of replication protein A. *J. Cell Biol.* **119**, 1-15.

Affolter, M., Cote, J., Renaud, J. and Ruiz-Carrillo, A. (1987). Regulation of histone and β^A-globin gene expression during differentiation of chicken erythroid cells. *Mol. Cell. Biol*. **7**, 3663-3672.

Allan, J., Cowling, G. J., Harborne, N., Cattini, P., Craigie, R. and Gould, H. (1981). Regulation of the higher-order structure of chromatin by histones H1 and H5. *J. Cell Biol*. **90**, 279-288.

Almouzni, G. and Mechali, M. (1988). Assembly of spaced chromatin involved of ATP and DNA topoisomerase activity. *EMBO J.* **7**, 4355-4365.

Aubert, D., Garcia, M., Benchaibi, M., Poncet, D., Chebloune, Y., Verdier, G., Nigon, V., Samarut, J. and Mura, C. V. (1991). Inhibition of proliferation of primary avian fibroblasts through expression of histone H5 depends on the degree of phosphorylation of the protein. *J. Cell Biol.* **113**, 497-506.

Benton B. M., Eng, W., Dunn, J. J., Studier, F. W., Sternglanz, R. and Fisher, P. A. (1990). Signal-mediated import of bacteriophage T7 RNA polymerase into the *Saccharomyces cerevisiae* nucleus and specific transcription of target genes. *Mol. Cell. Biol.* **10**, 353-360.

Bergman, M. G., Wawra, E. and Winge, M. (1988). Chicken histone H5 inhibits transcription and replication when introduced into proliferating cells by microinjection. *J. Cell Sci.* **91**, 201-209.

Blow, J. J. and Laskey, R. A. (1986). Initiation of DNA replication in nuclei and purified DNA by a cell-free extract of *Xenopus* eggs. *Cell* **47**, 577-587.

Blow, J. J. and Watson, J. V. (1987). Nuclei act as independent and integrated units of replication in a *Xenopus* cell-free DNA replication system. *EMBO J.* **6**, 1997-2002.

Blow, J. J. and Laskey, R. A. (1988). A role for the nuclear envelope in controlling DNA replication within the cell cycle. *Nature* **332**, 546-548.

Blow, J. J. and Sleeman, A. M. (1990). Replication of purified DNA in *Xenopus* egg extract is dependent on nuclear assembly. *J. Cell Sci.* **95**, 383-391.

Bouvet, P., Dimitrov, S. and Wolffe, A. P. (1994). Specific regulation of *Xenopus* chromosomal 5S rRNA gene transcription *in vivo* by histone H1. *Genes Dev.* **8**, 1147-1159.

Brown, D. T. and Sittman, D. B. (1993). Identification through overexpression and tagging of the variant type of the mouse H1e and H1c genes. *J. Biol. Chem.* **268**, 713-718.

Brown, D. T., Alexander, B. T. and Sittman, D. B. (1996). Differential effect of H1 variant overexpression on cell cycle progression and gene expression. *Nucl. Acids Res.* **24**, 486-493.

Chipev, C. C. and Wolffe, A. P. (1992). Chromosomal organization of *Xenopus laevis* oocyte and somatic 5S rRNA genes *in vivo*. *Mol. Cell. Biol.* **12**, 45-55.

Coleman, T. R., Carpenter, P. B. and Dunphy, W. G. (1996). The *Xenopus* Cdc6 protein is essential for the initiation of a single round of DNA replication in cell-free extracts. *Cell* **87**, 53-63.

Coverley, D., Downes, C. S., Romanowski, P. and Laskey, R. A. (1993). Reversible effects of nuclear membrane permeabilization on DNA replication: evidence for a positive licensing factor. *J. Cell Biol.* **122**, 985-992.

Cox, L. S. and Leno, G. H. (1990). Extracts from eggs and oocytes of *Xenopus*

laevis differ in their capacities for nuclear assembly and DNA replication. *J. Cell Sci.* **97**, 177-184.

Cox, L. S. (1992). DNA replication in cell-free extracts from *Xenopus* eggs is prevented by disrupting nuclear envelope function. *J. Cell Sci.* **101**, 43-53.

Dilworth S. M., Black, S. J. and Laskey, R. A. (1987). Two complexes that contain histones are required for nucleosome assembly in vitro: role of nucleoplasmin and N1 in *Xenopus* egg extracts. *Cell* **51**, 1009-1018.

Dimitrov, S., Almouzni, G., Dasso, M. and Wolffe, A. P. (1993). Chromatin transitions during early *Xenopus* embryogenesis: changes in histone H4 acetylation and in linker histone type. *Dev. Biol.* **160**, 214-227.

Dimitrov, S., Dasso, M. C. and Wolffe, A. P. (1994). Remodeling sperm chromatin in *Xenopus laevis* egg extracts: the role of core histone phosphorylation and linker histone B4 in chromatin assembly. *J. Cell Biol.* **126**, 591-601.

Dimitrov, S. and Wolffe, A. P. (1996). Remodeling somatic nuclei in *Xenopus laevis* egg extracts: molecular mechanisms for the selective release of histones H1 and $H1^0$ from chromatin and the acquisition of transcriptional competence. *EMBO J.* **15**, 5897-5906.

Dunn, J. J., Krippl, B., Bernstein, K., Westphal, H. and Studier, F. W. (1988). Targeting bacteriophage T7 RNA polymerase to the mammalian cell nucleus. *Gene* **68**, 259-266.

Dworkin-Rastl, E., Kandolf, H. and Smith, R. C. (1994). The maternal histone H1 variant, H1M (B4 protein), is the predominant H1 histone in *Xenopus* pregastrula embryos. *Dev. Biol.* **161**, 425-439.

Goldberg, M., Jenkins, H., Allen, T., Whitfield, W. G. F. and Hutchison, C. J. (1995). *Xenopus* lamin B_3 has a direct role in the assembly of a replication competent nucleus: evidence from cell-free egg extracts. *J. Cell Sci.* **108**, 3451-3461.

Grodberg, J. and Dunn, J. J. (1988). *ompT* encodes the *Escherichia coli* outer membrane protease that cleaves T7 RNA polymerase during purification. *J. Bacteriol.* **170**, 1245-1253.

Grunwald, D., Lawrence, J. and Khochbin, S. (1995). Accumulation of histone $H1^0$ during early *Xenopus laevis* development. *Exp. Cell Res.* **218**, 586-595.

Gurdon, J. B. (1968). Changes in somatic cell nuclei inserted into growing and maturing amphibian oocytes. *J. Embryol. Exp. Morph.* **20**, 401-414.

Halmer, L. and Gruss, C. (1995). Influence of histone H1 on the *in vitro* replication of DNA and chromatin. *Nucl. Acids Res.* **23**, 773-778.

Hock, R., Moorman, A., Fischer, D. and Scheer, U. (1993). Absence of somatic histone H1 in oocytes and preblastula embryos of *Xenopus laevis*. *Dev. Biol.* **158**, 510-522.

Hozak, P., Sasseville, A. M., Raymond, Y. and Cook, P. R. (1995). Lamin proteins form an internal nucleoskeleton as well as a peripheral lamina in human cells. *J. Cell Sci.* **108**, 635-644.

Hutchison, C. J., Cox, R. and Ford, C. C. (1988). The control of DNA replication in a cell-free extract that recapitulates a basic cell cycle *in vitro*. *Development* **103**, 553-566.

Hutchison, C. J. and Kill, I. (1989). Changes in the nuclear distribution of DNA polymerase alpha and PCNA/cyclin during the progress of the cell cycle, in a cell-free extract of *Xenopus* eggs. *J. Cell Sci.* **93**, 605-613.

Hutchison, C. J., Bridger, J. M., Cox, L. S. and Kill, I. R. (1994). Weaving a pattern from disparate threads: lamin function in nuclear assembly and DNA replication. *J. Cell Sci.* **107**, 3259-3269.

Jenkins, H., Holman, T., Lyon, C., Lane, B., Stick, R. and Hutchison, C. (1993). Nuclei that lack a lamina accumulate karyophilic proteins and assemble a nuclear matrix. *J. Cell Sci.* **106**, 275-285.

Kandolf, H. (1994). The H1A histone variant is an *in vivo* repressor of oocyte-type 5S gene transcription in *Xenopus laevis* embryos. *Proc. Nat. Acad. Sci. USA* **91**, 7257-7261.

Khochbin, S. and Wolffe, A. P. (1994). Developmentally regulated expression of linker-histone variants in vertebrates. *Eur. J. Biochem.* **225**, 501-510.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680-685.

Laskey, R. A., Mills, A. D. and Morris, N. R. (1977). Assembly of SV40 chromatin in a cell free system from *Xenopus* eggs. *Cell* **10**, 237-243.

Laskey, R. and Madine, M. (1996). Roles of nuclear structure in DNA replication. In *DNA Replication in Eukaryotic Cells* (ed. M. DePamphilis), pp. 119-130. Cold Spring Harbor Laboratory Press, USA.

Leng, F. and Leno, G. H. (1997). Daunomycin disrupts nuclear assembly and the coordinate initiation of DNA replication in *Xenopus* egg extracts. *J. Cell. Biochem.* **64**, 476-491.

Leno, G. H. and Laskey, R. A. (1991). The nuclear membrane determines the timing of DNA replication in *Xenopus* egg extracts. *J. Cell Biol.* **112**, 557-566.

Leno, G. H., Downes, C. S. and Laskey, R. A. (1992). The nuclear membrane prevents replication of human G2 nuclei, but not G1 nuclei, in *Xenopus* egg extract. *Cell* **69**, 151-158.

Leno, G. H. and Munshi, R. (1994). Initiation of DNA replication in nuclei from quiescent cells requires permeabilization of the nuclear membrane. *J. Cell Biol.* **127**, 5-14.

Leno, G. H., Mills, A. D., Philpott, A. and Laskey, R. A. (1996). Hyperphosphorylation of nucleoplasmin facilitates *Xenopus* sperm decondensation at fertilization. *J. Biol. Chem.* **271**, 7253-7256.

Leno, G. H. and Munshi, R. (1997). Reactivation of DNA replication in nuclei from terminally differentiated cells: nuclear membrane permeabilization is required for initiation in *Xenopus* egg extract. *Exp. Cell Res.* **232**, 412-419.

Lohka, M. J. and Masui, Y. (1983). Formation *in vitro* of sperm pronuclei and mitotic chromosomes induced by amphibian ooplasmic components. *Science* **220**, 719-721.

Lohka, M. J. and Masui, Y. (1984). Roles of cytosol and cytoplasmic particles in nuclear envelope assembly and sperm pronuclear formation in cell-free preparations from amphibian eggs. *J. Cell Biol.* **98**, 1222-1230.

Lohr, D., Corden, J., Tatchell, K., Kovacic, R. T. and van Holde, K. E. (1977). Comparative subunit structure of HeLa, yeast, and chicken erythrocyte chromatin. *Proc. Nat. Acad. Sci. USA* **74**, 79-83.

Lourim, D., Kempf, A. and Krohne, G. (1996). Characterization and quantitation of three B-type lamins in *Xenopus* oocytes and eggs: increase of lamin L_I protein synthesis during meiotic maturation. *J. Cell Sci.* **109**, 1775-1785.

Luderus, M. E., de Graf, A., Mattia, E., den Blaauwen, J. L., Grande, M. A., de Jong, L. and von Driel, R. (1992). Binding of matrix attachment regions to lamin B_1. *Cell* **70**, 949-959.

Matsuoka, Y., Takechi, S., Nakayama, T. and Toneda, Y. (1994). Exogenous histone H1 injection into mitotic cells disrupts synchronous progression of mitotic events by delaying chromosome decondensation. *J. Cell Sci.* **107**, 693-701.

Meier, J., Campbell, K. H. S., Ford, C. C., Stick, R. and Hutchison, C. J. (1991). The role of lamin L_{III} in nuclear assembly and DNA replication, in cell-free extracts of *Xenopus* eggs. *J. Cell Sci.* **98**, 271-279.

Mills, A. D., Blow, J. J., White, J. G., Amos, W. B., Wilcock, D. and Laskey, R. A. (1989). Replication occurs at discrete foci spaced throughout nuclei replicating in vitro. *J. Cell Sci.* **94**, 471-477.

Moir, R. D., Montag-Lowy, M. and Goldman, R. D. (1994). Dynamic properties of nuclear lamins: lamin B is associated with sites of DNA replication. *J. Cell Biol.* **125**, 1201-1212.

Murphy, A. W., Crompton, C. M., Hainey, S., Codd, G. A. and Hutchison, C. J. (1995). The role of protein phosphorylation in the assembly of a replication competent nucleus: investigations in *Xenopus* egg extracts using the cyanobacterial toxin microcystin-LR. *J. Cell Sci.* **108**, 235-244.

Murray, A. W. and Kirschner, M. W. (1989). Cyclin synthesis drives the early embryonic cell cycle. *Nature* **339**, 275-280.

Nelson, P. P., Albright, S. C., Wiseman, J. M. and Garrard, W. T. (1979). Reassociation of histone H1 with nucleosomes. *J. Biol. Chem.* **254**, 11751-11760.

Newport, J. and Kirschner, M. (1982). A major developmental transition in early *Xenopus* embryos: I. characterization and timing of cellular changes at the midblastula stage. *Cell* **30**, 675-686.

Newport, J. (1987). Nuclear reconstitution in vitro: stages of assembly around protein-free DNA. *Cell* **48**, 205-217.

Newport, J. W., Wilson, K. L. and Dunphy, W. G. (1990). A lamin-independent pathway for nuclear envelope assembly. *J. Cell Biol.* **111**, 2247-2259.

Nightingale, K., Dimitrov, S., Reeves, R. and Wolffe, A. P. (1996). Evidence for a shared structural role for HMG1 and linker histones B4 and H1 in organizing chromatin. *EMBO J.* **15**, 548-561.

Philpott, A., Leno, G. H. and Laskey, R. A. (1991). Sperm decondensation in *Xenopus* egg cytoplasm is mediated by nucleoplasmin. *Cell* **65**, 569-578.

Philpott, A. and Leno, G. H. (1992). Nucleoplasmin remodels sperm chromatin in *Xenopus* egg extracts. *Cell* **69**, 759-767.

Rodriguez-Campos, A., Shimamura, A. and Worcel, A. (1989). Assembly and properties of chromatin containing histone H1. *J. Mol. Biol.* **209**, 135-150.

Sheehan, M. A., Mills, A. D., Sleeman, A. M., Laskey, R. A. and Blow, J. J. (1988). Steps in the assembly of replication-competent nuclei in a cell-free system from *Xenopus* eggs. *J. Cell Biol.* **106**, 1-12.

Shimamura, A., Sapp, M., Rodriguez-Campos, A. and Worcel, A. (1989). Histone H1 represses transcription from mini chromosomes assembled *in vitro*. *Mol. Cell. Biol.* **9**, 5573-5584.

Smith, R. C., Dworkin-Rastl, E. and Dworkin, M. B. (1988). Expression of a histone H1-like protein is restricted to early *Xenopus* development. *Genes Dev*. **2**, 1284-1295.

Sun, J., Wiaderkiewicz, R. and Ruiz-Carrillo, A. (1989). Histone H5 in the control of DNA synthesis and cell proliferation. *Science* **245**, 68-71.

Tremethick, D. J. and Frommer, M. (1992). Partial purification, from *Xenopus laevis* oocytes, of an ATP-dependent activity required for nucleosome spacing *in vitro*. *J. Biol. Chem.* **267**, 15041-15048.

Trieschmann, L., Alfonso, P. J., Crippa, M. P., Wolffe, A. P. and Bustin, M. (1995). Incorporation of chromosomal proteins HMG-14/HMG-17 into nascent nucleosomes induces an extended chromatin conformation and enhances the utilization of active transcription complexes. *EMBO J.* **14**, 1478-1489.

Ura, K., Hayes, J. J. and Wolffe, A. P. (1995). A possible role for nucleosome mobility in the transcriptional activity of chromatin templates: restriction by linker histones. *EMBO J.* **14**, 3752-3765.

Ura, K., Nightingale, K. and Wolffe, A. P. (1996). Differential association of HMG1 and linker histones B4 and H1 with dinucleosomal DNA: structural transitions and transcriptional repression. *EMBO J.* **15**, 4959-4969.

Wolffe, A. P. (1989). Dominant and specific repression of *Xenopus* oocyte 5S RNA genes and satellite I DNA by histone H1. *EMBO J*. **8**, 527-537.

Zawadzki, V. and Gross, H. J. (1991). Rapid and simple purification of T7 RNA polymerase. *Nucl. Acids Res.* **19**, 1948.

Zhang, C., Jenkins, H., Goldberg, M. W., Allen, T. D. and Hutchison, C. J. (1996). Nuclear lamina and nuclear matrix organization in sperm pronuclei assembled in *Xenopus* egg extract. *J. Cell Sci.* **109**, 2275-2286.

Zhao, K., Kas, E., Gonzalez, E. and Laemmli, U. K. (1993). SAR-dependent mobilization of histone H1 by HMG-I/Y *in vitro* : HMG-I/Y is enriched in H1-depleted chromatin. *EMBO J.* **12**, 3237-3247.

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