Stability of the conservative mode of nucleosome assembly

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SUMMARY

The conservative assembly of nucleosome histone octamer cores has been confirmed by electrophoretic analysis of density labeled histones following equilibrium buoyant density centrifugation. After normal replication, crosslinked octamers are shown not to contain a mixture of new and old core histones. Moreover, when DNA synthesis is inhibited by ara-C nucleosome cores are still assembled exclusively from nascent histone. Similarly, after release from cycloheximide inhibition newly synthesized core histone is conservatively deposited. Thus, a conservative mechanism of histone octamer assembly occurs when nascent histone is present in the normal stoichiometry to nascent DNA and when chromatin is assembled in nascent histone or nascent DNA excess.

INTRODUCTION

Heterogeneity in the composition and conformation of chromosomal proteins has been linked to functional differences between nucleosomes (1,2). Several studies have demonstrated the existence of distinct classes of nucleosomes varying in histone primary amino acid sequence (3,4), histone covalent modification (5) or nonhistone complement (6-8) within a single cell type.

The maintenance of specifically constituted nucleosomes on defined regions of chromatin by the nonrandom assembly and segregation of chromosomal proteins provides a possible mechanism for the propagation of phenotypic information over successive cell generations (9). Using density labeling and equilibrium buoyant density centrifugation it was previously shown that essentially all the nascent core histones are conservatively deposited and segregated in nucleosomes (9,10). A similar conclusion was reached by Prior et al. (11) who showed that exogenous histone H3 molecules taken up by *Physarum* microplasmodia are conservatively assembled and segregated in nucleosomes. Consistent with these observations are the findings that nascent core histones bind preferentially to newly synthesized DNA (12-15), and that parental histones segregate to one arm of the replication fork, (the leading side for DNA synthesis) during cycloheximide incubation (16-20). Additional studies suggest

that at least 5-22% of the newly made core histones segregate with the newly made DNA over successive generations (21).

Nevertheless, there remains some disagreement in the literature regarding the conservative mode of nucleosome assembly (15,18,22-27). The present study represents a reexamination of nucleosome assembly in MSB cells (28), using density labeling and isopycnic centrifugation coupled with a gel electrophoretic analysis of the banding patterns of particular crosslinked histone particles. The results show that nascent core histone octamers are uniformly dense (i.e. contain only newly synthesized histone) while core octamers crosslinked to H1 and nonhistone proteins are of hybrid density. Upon removal of the H1 and nonhistone proteins and subsequent crosslinking, all octamer cores band as uniformly dense particles. Therefore, under normal growth conditions nucleosome cores are assembled conservatively.

This work has been extended to examine the mode of nucleosome assembly under conditions where the normal process of chromatin replication is perturbed. When MSB cells are incubated with 10^{-4} M cytosine arabinoside (ara-C) nucleosome core histones are produced in stoichiometric proportion in approximately 100-200 fold excess over nascent DNA (I.M.L., submitted). Under these conditions, histone octamers are conservatively assembled. Alternatively, when MSB cells are incubated with 50 μ M cycloheximide, DNA synthesis proceeds at approximately 20% of control rates, in the absence of histone synthesis. Upon release of the cycloheximide block there is a burst of histone synthesis and the histone-depleted DNA becomes incorporated into nucleosomes. When the histones synthesized during the drug recovery interval are labeled with density substituted amino acids, crosslinked and resolved by isopycnic centrifugation, the nascent octamer cores band as uniformly dense particles. Therefore, the conservative mode of nucleosome core assembly is maintained under conditions of nascent histone or nascent DNA excess and appears to be a fundamental aspect of chromatin replication.

METHODS

Cell Culture:

MSB-1 Cells (28) were grown in RPMI-1640 supplemented with 5% fetal calf serum plus 5% calf serum, and gentamycin (Schering) at 40°C in a humidified 10% CO₂ atmosphere. All experiments were performed on exponentially growing cultures, normally 5×10^5 - 10^6 cells/ml. Under these conditions the cell number doubled in approximately 10 hours, and contained greater than 93% viable cells (by trypan blue exclusion). Cultures were routinely monitored for mycoplasma contamination.

Labeling:

Cells were routinely washed with warmed phosphate-buffered saline, concentrated approximately 20-fold and suspended in warmed density substituted medium. The density labeling medium contained ^{15}N , ^{13}C , ^2H substituted amino acids (Merck MB1808) filtered through an Amicon UM2 membrane and reconstituted to approximate RPMI-1640. After ten minutes at 40°C ^3H -lysine (NEN, 75 Ci/mmol, 100-200 $\mu\text{Ci/ml}$) was added for an additional 50 minutes. Where indicated, ara-C or cycloheximide were present at 100 μM or 50 μM respectively.

Chromatin Preparation and Isopycnic Centrifugation:

Cells in culture were washed with PBS and lysed in 0.5% Nonidet P-40, 10 mM NaCl, 5 mM Pipes (pH 7.4), 3 mM MgCl_2 and the nuclei washed twice in this buffer. Nuclei were resuspended in cell lysis buffer and digested mildly (5-10% acid solubility) with micrococcal nuclease (Worthington or Boehringer) at 5 enzyme units/ A_{260} unit for 5 minutes (37°C digest) after the addition of CaCl_2 to 0.1 mM. Digestion was stopped by the addition of EGTA to 0.1 mM and chilling. Pelleted nuclei were lysed in 10 mM Na_2EDTA (pH 7.4) and the soluble chromatin (25-35% of the initial A_{260}) was made 0.65 M in NaCl and 20 mM in $\text{Na}_2\text{B}_4\text{O}_7$ (pH 9.0). The chromatin was mixed with 3 volumes of BioRad AG50W-X4 resin (equilibrated with the same buffer) and shaken at 4°C for 30 minutes. The resin was pelleted and an aliquot of the H1-, nonhistone-depleted chromatin supernatant was crosslinked by adding a stock of dithiobissuccinimidyl propionate (Pierce) (5 mg/ml in DMSO) to a final concentration of 0.5 mg/20 A_{260} . The crosslinked chromatin (85 μl) was mixed with 300 μl of 2.25 M cesium formate (CsFo), 5.8 M guanidine hydrochloride (GuCl), 15 μl of uncrosslinked, depleted chromatin was added and the mixture underlayered beneath 1.7 ml of 0.93 M CsFo, 6.3 M GuCl. The sample was overlaid with paraffin oil and centrifuged to equilibrium at 25°C in the Beckman SW60 Ti rotor at 45,000 rpm for 68 hours. Routinely, 100-200 μg of chromatin was loaded per gradient. The gradients were fractionated from below and microdialyzed against SDS-PAGE sample buffer omitting β -mercaptoethanol at all times (29,30) and electrophoresed until histone standards (fluorescently labeled with 2-methoxy-2,4-diphenyl-3(2H)furanone (MDPF) and visualized with a hand-held UV light) migrated to within 1 cm of the bottom of the gel (42). MDPF was the generous gift of Dr. W. Scott, Hoffman-LaRoche. Gels were processed for fluorography using Enhance (NEN) and exposed to pre-flashed (43) Kodak XR-5 film at -70°C .

RESULTS

Conservative Deposition of Core Histones:

During chromatin replication, do newly made histones mix with preexisting histones in individual nucleosomes? We had previously approached this question by measuring the density of crosslinked nucleosomal histones synthesized in the presence of density labeled amino acids (10) and found that the assembly of nucleosomes is nonrandom, i.e., newly synthesized and parental histones are not found in the same nucleosome. To demonstrate this point in a more graphic fashion that might also permit a more detailed description of the process of nucleosome assembly we have used MSB cells, which are chick lymphoid leukemic cells transformed by Marek's virus (28). Under our conditions, these cells incorporate a greater percentage of dense amino acids into protein than the chick myoblasts used previously (10). MSB cells were incubated for 1 hour in tissue culture medium substituted simultaneously with dense amino acids and ^3H -lysine. Nuclei were isolated and nucleosomes were prepared by moderate digestion with staphylococcal nuclease. An aliquot of the sample was crosslinked using dithiobissuccinimidyl propionate (SP), mixed with an uncrosslinked aliquot of the same chromatin in the presence of cesium formate and guanidine hydrochloride, and centrifuged to equilibrium on a denaturing cesium formate-guanidine hydrochloride density gradient. Under these conditions the DNA and histones (crosslinked and uncrosslinked) dissociate, and the histones equilibrate as free protein while the denser DNA pellets. An identical gradient containing trace amounts of ^3H -labeled, dense, uncrosslinked histones and ^{14}C -labeled, light, crosslinked histones was run in parallel to provide external markers. Fractions were collected from the marker gradient and counted directly while fractions from the primary gradient were microdialysed and electrophoresed on SDS-polyacrylamide gels (29,30). The Coomassie blue stained gel of Figure 1A shows the distribution of unlabeled histone. It can be seen that both the crosslinked and uncrosslinked inner histones form bands which peak in the same region of the gradient as the light marker. We note in these and other similar gradients that several chromosomal nonhistone proteins (as well as exogenous BSA; not shown) peak slightly denser than the nucleosomal core histones, while H1 peaks 1-2 fractions lighter. These differences are independent of substitution with dense amino acids and presumably reflect a differential affinity for Cs^+ ion (31). The fluorogram of this gel (Figure 1B) shows the distribution of ^3H -lysine labeled histones in the same gradient. The bands of crosslinked histone octamers and 16-mers peak in the same region as the fully dense marker and the uncrosslinked, fully

Figure 1. Assembly of Nucleosomal Histones.

Exponentially growing cells were labeled for one hour with ^3H -lysine in density-substituted medium, a 10-fold excess of unlabeled cells was added, chromatin prepared and 80% of the sample ($A_{260} = 20$) crosslinked at 2.5 mg/ml SP, 20% crosslinked at 0.25 mg/ml SP. The samples were mixed with CsFo-GuCl and combined prior to equilibrium density centrifugation. (A) Coomassie stained 5-22% gradient polyacrylamide-SDS gel showing the banding pattern of light (normal density) proteins. (B) Fluorogram of (A) showing the density distribution of the tritiated proteins. DENSE and LIGHT represent peak positions of uncrosslinked, tritiated, density-substituted ($\rho = 1.30 \text{ gm/ml}$) and crosslinked, ^{14}C -labeled, light proteins, ($\rho = 1.24 \text{ gm/ml}$) run in a parallel gradient.

dense histones contained in the same gradient; however, close examination of Figure 1B shows that the octamer band is actually a doublet and that the larger crosslinked particles may be slightly less dense than true histone octamers. In agreement with the results of Reudelhuber et al. (32), two-dimensional gel analysis and crosslinking experiments performed at 0.65 M NaCl (not shown) indicated that the larger particles contain H1 crosslinked to the core octamer. This slight shift in density suggests that dense octamers become associated with light H1 molecules; however, because H1 represents approximately 17% of

Figure 2. Conservative Assembly of Core Histone Octamers.

Exponentially growing cells were density labeled as in Figure 1 and chromatin prepared. The chromatin was depleted of H1 and nonhistones in 0.65 M NaCl in the presence of BioRad AG50W-X4 resin (Methods). An aliquot (~80%) was crosslinked at 0.5 mg SP/mg DNA, and mixed with uncrosslinked, depleted chromatin in the presence of CsFo-GuCl. The mixture was centrifuged to equilibrium on a CsFo-GuCl gradient and fractions desalted, followed by SDS-PAGE. (A) Coomassie stained gel. (B) Fluorogram of (A). Righthand lane (marked -XL) represents the protein pattern of the uncrosslinked depleted chromatin prior to centrifugation.

this particle by weight, a crosslinked light H1-dense octamer might not be completely resolved from totally dense octamers. In order to examine the assembly of the nucleosome core apart from the deposition of histone H1 the

same density labeling protocol was followed as described above except that the chromatin was depleted of H1 (and several nonhistone proteins) by incubation at 0.65 M NaCl in the presence of a cation exchange resin prior to crosslinking. The results obtained after isopycnic centrifugation of this material are shown in Figure 2. The right hand (marker) lane of the gel (Figure 2) shows that H1 is approximately 80% depleted in the 0.65 M NaCl supernatant after removal of the cation exchange resin. The remaining lanes of Figure 2A show the distribution of the bulk (unlabeled) proteins in the gradient. The fluorogram of Figure 2B shows that all of the crosslinked histone octamers band as uniformly dense particles. (Similar results are obtained when the density labeled chromatin is crosslinked in the presence of 1.0 M NaCl or after mild trypsin treatment [not shown]). A small percentage (10-20%) of the 16-mer species is shifted towards hybrid density and may represent a special population of adjacent nucleosomes.

The conclusion that nucleosomal histones assemble conservatively rests on our ability to distinguish particles which are completely dense from those of hybrid density. In order to unambiguously demonstrate this point the following reconstitution experiment was performed. MSB cells were pulsed with ^3H -lysine in the presence of dense amino acids for three hours, mixed with a 10-fold excess of unlabeled cells, chromatin isolated, dissociated in 2.5 M NaCl/6 M urea, dialysed directly to 2.0 M NaCl and by gradient to 0.7 M NaCl. The reconstitute was then mildly crosslinked with SP to yield core histone dimers, trimers and tetramers. (Crosslinked products were characterized by second-dimension electrophoresis after crosslink reversal by β -mercaptoethanol; not shown). The stained gel of Figure 3A shows that the prominent histone dimer band peaks at the light density position. The ^3H -labeled dimer (Figure 3B) is shifted to a hybrid density position midway between dense and light however, as expected for a reconstituted heavy-light product. (Semihistone A24 also bands with hybrid density due to the *in vivo* conjugation of light ubiquitin with dense H2A, and vice versa [33]). Longer exposure of the fluorogram (Figure 2C) reveals the ^3H -labeled trimer and tetramer peaking at progressively lighter positions. Thus, these gradients are able to distinguish products which are approximately one-third dense (trimers) from those which are half dense (dimers) or one-fourth dense (tetramers). In agreement with our earlier report (10) the above results confirm the observation of nonrandom, conservative assembly of the nucleosomal core histones.

Figure 3. Density Gradient Sensitivity.

Cells were labeled with ^3H -lysine for 3 hours in dense medium and mixed with a 10-fold excess of unlabeled cells. Chromatin was prepared, dissociated in high salt/urea and gradient reconstituted. The reconstitute was mildly crosslinked with SP and centrifuged on CsFo-GuCl. (A) Stained 15% gel showing banding pattern of unsubstituted proteins. (B) Fluorogram of (A) showing distribution of ^3H -containing crosslinked and uncrosslinked proteins. (C) Four-fold reexposure of (B). The positions of DENSE ($\rho = 1.30 \text{ gm/ml}$) and LIGHT ($\rho = 1.24 \text{ gm/ml}$) parallel histone markers are indicated.

The high molecular weight material at the top of the separating gel equilibrates over a wide range of density, contrary to expectation. This is attributable to its content of both crosslinked histone and uncrosslinked high molecular weight nonhistone proteins, as demonstrated by two dimensional electrophoresis (not shown).

Conservative Assembly of Core Histone Octamers During Replication in ara-C:

During incubation of MSB cells in 10^{-4} M ara-C nascent core histones are deposited stoichiometrically in the nucleus at approximately 20% of control rates, while DNA synthesis is inhibited by more than 99.8% (I.M.L., submitted).

This system thus affords the opportunity to study nucleosome assembly in the presence of a 100-200 fold excess of nascent histone to nascent DNA. Recent reports (34-36) have suggested that free histone octamer cores can associate with octamer cores residing on DNA. In light of the observation that excess histone is bound per unit length of DNA during replication in ara-C (I.M.L., submitted), it was of interest to determine whether the conservative mode of nucleosome deposition persists during replication in the presence of ara-C or whether new and old histones become mixed in individual nucleosome core octamers.

MSB cells were preincubated in ara-C for one hour and transferred to density labeling medium containing ara-C and ^3H -lysine. After one hour, approximately 5% of the total cell histones have been labeled. Chromatin was isolated by micrococcal nuclease solubilization and depleted of nonhistones and histone H1 as above, to allow examination of the nucleosome core histones exclusively. An aliquot of the chromatin was crosslinked with SP, remixed with an uncrosslinked aliquot of the same chromatin in the presence of cesium formate and guanidine hydrochloride and centrifuged to equilibrium as above. The Coomassie stained gel (Figure 4A) reveals the banding pattern of the preexisting, unsubstituted (light) protein in the density gradient, while the fluorogram of this gel (Figure 4B) shows the distribution of the nascent histones. It is seen that the ^3H -labeled histone octamers band in the fully dense region of the gradient, as defined by the position of the internal markers, the uncrosslinked monomer histones. Thus, the nascent octamers deposited during ara-C inhibition appear to have been conservatively assembled exclusively from dense histones.

Conservation Deposition of Core Histones During Recovery from Cycloheximide:

Seale and Simpson (37) and Weintraub (16,17) have reported that incubation of cells at appropriate levels of cycloheximide leads to a rapid cessation of histone synthesis, while DNA synthesis continues with linear kinetics at approximately 20% of control rates for over one hour. Nuclease digestion (16-18,20) of the DNA synthesized during the cycloheximide incubation has suggested that the parental histones are retained as intact nucleosomes on one

Figure 4. Conservative Assembly of Histone Octamers during Ara-C Incubation.

MSB cells growing in suspension were preincubated with ara-C (50 µg/ml) for 40 minutes and transferred (at 10^8 cells/ml) to density substituted amino acid medium containing 50 µg/ml ara-C. After 10 minutes to chase light amino acids from cellular pools, ³H-lysine was added (New England Nuclear, 75 Ci/mole, 200 µCi/ml) and incubation continued for 40 minutes. Chromatin was prepared, resin-depleted of H1 and nonhistones, crosslinked and centrifuged to equilibrium on a CsFo-GuCl gradient as described. Fractions were desalted and analysed by 5-22% acrylamide gradient SDS-PAGE. (A) Stained gel. (B) Fluorogram of (A).

arm of the replication fork (the leading arm for replication [20]) leaving the other arm virtually devoid of histones. Thus the segregation of parental histone is conservative (19), implying that the deposition of nascent histone

during the recovery from cycloheximide might also be conservative. By density labeling of the histones synthesized during the recovery phase, it is possible to test this hypothesis directly. When MSB cells are incubated with 50 μ M cycloheximide, nascent histone synthesis is inhibited to a greater extent than nascent DNA synthesis (Figure 5A). This differential inhibition is reflected in an increased sensitivity of the nascent DNA to nuclease digestion (Figure 5B), and has been interpreted as the result of an absence of histone on one arm of the replication fork. Consistent with the observations in other cell types (16,37) when MSB cells are released from cycloheximide inhibition, there is a rapid increase in histone synthesis and histones are deposited on chromatin at approximately 2-3 times the rate required by concomitant DNA synthesis (Figure 5A). During this chase, the DNA synthesized previously in the presence of cycloheximide largely acquires the resistance to nuclease digestion (Figure 5B) of control chromatin. Analysis by sucrose gradient sedimentation and gel electrophoresis also indicate that during a drug-free chase the DNA synthesized in the presence of cycloheximide is incorporated into normal nucleosomes (not shown).

To examine nucleosome assembly during the recovery from cycloheximide, MSB cells were preincubated briefly with cycloheximide in density substituted medium and transferred to drug-free density substituted medium containing 3 H-lysine. After a 30 minute incubation, chromatin was isolated, depleted of nonhistones and histone H1 and a mixture of crosslinked and uncrosslinked chromatin centrifuged to equilibrium on a CsFo-GuCl gradient as above. After centrifugation the gradient was fractionated and the fractions analyzed by SDS-PAGE. The fluorogram of Figure 6 shows that the 3 H-labeled crosslinked histone octamers band as uniformly dense particles. Therefore, there has been no detectable mixing of new and old histone in these octamers.

DISCUSSION

The conservative assembly and segregation of nucleosome cores represents a means whereby information controlling gene structure and expression can be stably incorporated into chromatin. The present data suggest that density labeled nascent core histones are assembled to form octamers containing exclusively nascent molecules. Our previous controls (10) and the experiment of Figure 3 demonstrate that the isopycnic centrifugation/SDS-PAGE technique has the sensitivity to distinguish completely dense octamers from hybrid octamers containing three or more light histones. In theory, randomization of octamer assembly to the extent that one or two parental histones (e.g. an H2A-H2B pair)

Figure 5. Cycloheximide Effects on Chromatin Synthesis and Structure.

(A) CHX, MSB cells were incubated with cycloheximide (50 μ M) and a mixture of 14 C-thymidine (1 μ Ci/ml) and 3 H-lysine (100 μ Ci/ml) for 30 minutes; CON, cells were incubated with label as above, but in the absence of cycloheximide; REC, cells were preincubated with cycloheximide for 30 minutes, washed free of drug and incubated in a drug-free conditioned medium with the label mixture for an additional 30 minutes. 14 C-thymidine incorporation was determined by TCA precipitation of an aliquot of nuclei from each cell incubation. 3 H-lysine incorporation into histone was measured by SDS-PAGE of the acid-soluble protein from a separate aliquot of nuclei and excision and scintillation counting of histone bands. All 3 H counts have been corrected for 14 C spill. (B) Cells were labeled with 3 H-thymidine (5 μ Ci/ml) for 30 minutes in the absence of drug (Δ) or in the presence of (50 μ M) cycloheximide ($x, \circ$). One aliquot of cells was harvested immediately after the cycloheximide incubation ($\circ$), while another was washed and incubated in drug-free, conditioned medium for an additional 30 minutes (x). Nuclei were isolated as in Figure 1, and digested with 10 units of micrococcal nuclease per A₂₆₀ unit at 37°C. At the indicated times, aliquots were mixed with 10 mM Na₂EDTA (pH 7.5), 0.1% SDS, 200 μ g/ml protease K and incubated on ice for 5 minutes and at 37°C for 30 minutes. TCA-precipitable material was collected on glass fiber filters and the percent 3 H-thymidine solubilized calculated after treatment of the filters with Protosol (NEN) and scintillation counting in Beckman Ready-Solv MP.

Figure 6. Conservative Deposition of Core Histones During Recovery from Cycloheximide.

Cells were preequilibrated with density substituted (dense) medium for 10 minutes and cycloheximide was added to 50 μ M for an additional 30 minutes. The cells were washed with PBS and resuspended in drug-free dense medium containing 3 H-lysine (200 μ Ci/ml) for 30 minutes. The cells were harvested and subsequent chromatin preparation, isopycnic centrifugation and SDS-PAGE were as in Figure 2. [Control experiments (not shown) have demonstrated that the histones deposited during the 30 minute recovery interval are quantitatively the same in their crosslinking pattern as control histones.] Fluorogram is shown.

were assembled into an otherwise dense octamer would require that 12-25% of the 3 H-octamers would be of the complementary one dense plus seven light histone composition, and would band very near the light marker. Overexposure of the fluorograms show no indication of such predominantly light, hybrid particles however. Furthermore, we have recently shown that core octamers assembled during a density pulse (in the presence or absence of ara-C) are nonrandomly segregated over more than 4-5 successive replications in normal medium (I.M.L., submitted). This biological demonstration of nucleosome stability argues strongly that nascent nucleosome cores are assembled by a completely conservative mechanism.

Electrophoretic analysis of gradients containing crosslinked proteins derived from density labeled chromatin indicate that while the nucleosome core is conservatively assembled, preexisting H1 or nonhistone proteins may become associated with nascent cores. However, the experiments presented are not designed to distinguish between random and semiconservative modes of

association of these proteins with the nascent core. (Indeed, this data cannot exclude possible artifactual randomization of H1 or nonhistone during chromatin preparation.) The interaction of new and old proteins within a single nucleosome may explain the discrepancies in interpretation of the data on nucleosome assembly and inheritance from several laboratories (10,21,23,26).

Several reports have suggested that nucleosomes are capable of binding excess histones (34-36). When MSB cells assemble chromatin in the presence of excess histone (during ara-C incubation) no mixing of new (density labeled) and old (light) histones is evident. Therefore, if new and old octamers interact under these conditions, this interaction does not seem to result in a mixing of the two histone populations in individual nucleosomes.

During cycloheximide incubation, MSB cells synthesize histone-depleted DNA and upon drug recovery, therefore, histones are deposited in the nucleus in the presence of an excess of free DNA. Although the present results do not directly address the question of the site of deposition of the nascent histone, it is clear that nascent octamers are conservatively assembled under these conditions.

Several laboratories have inferred that the process of chromatin assembly is nonrandom in terms of the sequence (13,14) and sites (15-20,25,26,38,39) of histone deposition, and it has been suggested that the processes of nucleosome assembly and gene activation are temporally correlated (40,41). The present results are consistent with our own (10) and other (11,26) observations of the conservative assembly of nucleosomes and demonstrate that this nonrandom mode of histone deposition is a basic feature of chromatin replication. Further work (I.M.L., submitted) also suggests that once a nucleosome octamer core has been conservatively assembled it is stably inherited (segregated) during successive replications.

An attribute of the isopycnic centrifugation/PAGE technique is that it allows the independent examination of specific crosslinked protein products. Thus, variations on this approach may be used to monitor the deposition and inheritance of ancillary nucleosomal proteins, such as the HMGs. These experiments are currently in progress.

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