

Dynamics of the Genome during Early *Xenopus laevis* Development: Karyomeres As Independent Units of Replication

Jean-Marc Lemaître, Gérard Géraud, and Marcel Méchali

Institut Jacques Monod, Centre National de la Recherche Scientifique (CNRS), Laboratoire d'Embryologie Moléculaire, 75251 Paris Cedex 05, France

Abstract. During *Xenopus laevis* early development, the genome is replicated in less than 15 min every 30 min. We show that during this period, DNA replication proceeds in an atypical manner. Chromosomes become surrounded by a nuclear membrane lamina forming micronuclei or karyomeres. This genomic organization permits that prereplication centers gather on condensed chromosomes during anaphase and that DNA replication initiates autonomously in karyomeres at early telophase before nuclear reconstruction and mitosis completion. The formation of karyomeres is not dependent on DNA replication but requires mitotic spindle formation and the normal segregation of chromosomes. Thus, during early development, chromo-

somes behave as structurally and functionally independent units. The formation of a nuclear envelope around each chromosome provides an *in vivo* validation of its role in regulating initiation of DNA replication, enabling the rate of replication to accelerate and S phase to overlap M phase without illegitimate reinitiation. The abrupt disappearance of this atypical organization within one cell cycle after thirteen divisions defines a novel developmental transition at the blastula stage, which may affect both the replication and the transcription programs of development.

Key words: DNA replication • cell cycle • chromosomes • nuclear envelope • development

IN all eukaryotic cells the replication and segregation of chromosomes is highly coregulated. Each DNA sequence must be replicated only once during each cell cycle. Chromosomes must segregate only after every sequence has been duplicated, and the next round of duplication of chromosomes must not occur until segregation is completed. The tight coordination between mitotic division and cell growth observed in somatic cells ensures that S phase does not start before a crucial regulatory point is reached in the G1 period of the cell cycle (11, 46). A second checkpoint control occurs in G2 when the decision to segregate the duplicated chromosomes occurs. A different strategy is used during the rapid cleavages stages of several animal species, where an uncoupling of cell growth and cell division is observed. This is the case in *Xenopus laevis*, where maternal components necessary for cell divi-

sion have been stored in large excess in the oocyte. Relaxed from the constraint of growth, early embryonic cell cycles rapidly oscillate between S and M phases. A cytoplasmic cell cycle clock independent of the nucleus governs these oscillations (15, 17). Consequently, a highly ordered and punctual timing of S and M phase must occur so that the organization of the nucleus for division keeps pace with this cytoplasmic oscillation.

For some animal species, ultrastructural studies have revealed substantial differences in nuclear assembly between embryonic and somatic cells. Structures called karyomeres have been observed in sea urchin (33), polychaete (12), and *Xenopus* embryos (37), but the role played by this peculiar organization and its functional significance has not been explained. The assembly of the membrane-lamina and proteins involved in initiation and elongation of DNA synthesis was examined *in vivo* during the early cleavage stages within karyomeres. We investigated whether they could be involved in the regulation of DNA replication and its coupling to mitosis. We show here that karyomeres behave as independent units of DNA replication during early development. Formation of the karyomeres requires the formation of the mitotic spindle and segregation of chromosomes. The assembly of prereplica-

Address all correspondence to M. Méchali, Institut de Génétique Humaine, CNRS, Laboratoire Dynamique du Génome et Développement, 141 rue de la Cardonille, 34096 Montpellier, Cedex 05 France. Tel.: (33) 4-99-61-99-17. Fax: (33) 4-99-61-99-20. E-mail: mechali@igh.cnrs.fr

The present address of all authors is Institut de Génétique Humaine, CNRS, Laboratoire Dynamique du Génome et Développement, 141 rue de la Cardonille, 34096 Montpellier, Cedex 05 France.

tion complexes occurs on individual chromosomes as early as anaphase stage and the formation of the lamina occurs around the chromosomes well before the reconstruction of the nucleus, allowing DNA replication to proceed when chromosomes are not yet assembled together. This specific organization permits the S phase to largely overlap with the classical mitotic stage. We have also developed a new *in vitro* extract from early *Xenopus* embryos that reproduces both this dynamics of assembly of the nucleus observed *in vivo* and the DNA replication occurring during early embryonic cell divisions.

Materials and Methods

Xenopus Embryos

Embryos were grown in 0.1× Barth's medium as described (28). When necessary, DNA replication was blocked by a 45-min incubation in 0.1× Barth's medium containing 150 μg/ml aphidicolin. G2/prophase-synchronized embryos were obtained by 45 min incubation in 0.1× Barth's medium containing 150 μg/ml cycloheximide.

Preparation of *Xenopus* Egg and Embryo Extracts

The interphase egg extract was prepared as described (7). Metaphase extracts refer to cytotostatic factor (CSF) extracts (38), except that the CSF extract was prepared at 4° instead of 16°C.

Embryo extracts containing synchronized G2/prophase nuclei were prepared from 1,024-cell stage embryos. Embryos were dejellied with 2% cysteine HCl, pH 7.9, at the 256-cell stage and then incubated at the 512 cell-stage in 0.1× Barth's medium containing 150 μg/ml cycloheximide during 45 min. After several washes in 0.1× Barth's medium, embryos were homogenized through a 1-ml Gilson tip (Gilson, France) and centrifuged at 4°C for 10 min at 10,000 g. The supernatant was collected, avoiding the top lipid layer. In this condition, the endogenous embryonic nuclei remain in the supernatant. The extract was further supplemented with 8% glycerol, aliquoted, and then stored at −80°C. Progression of G2/prophase nuclei to mitosis was obtained by mixing 2 vol of the embryo extract with 8 vol of metaphase-arrested egg extract. Under these conditions, the nuclear envelope breaks down after a 30-min incubation at 23°C. Metaphase arrest was checked by microscopic examination of aliquots stained with Hoechst 33258 (Sigma Chemical Co., St. Louis, MO). Completion of mitosis and entry into S phase was induced by addition of 0.6 mM CaCl₂ at that stage.

Immunocytochemistry

Extracts containing nuclei were diluted 10-fold with fixation buffer (FB)¹ containing 50 mM KCL, 2% sucrose, 5 mM MgCl₂, 0.5 mM EDTA, 4% formaldehyde, 20 mM Hepes, pH 7.6. After a 30-min incubation at room temperature, the nuclei were overlaid on a 0.6-M sucrose cushion and then centrifuged onto glass coverslips. Samples were then treated for immunocytochemistry analysis. Alternatively, samples were placed on slides and directly fixed with an equal volume of 2× FB containing 1 μg/ml Hoechst 33258. Immunocytochemistry on embryos was carried out by removing dejellied embryos at different stages after fertilization that were then placed on coverslips. The medium was carefully removed and 5 μl of FB without sucrose was added to cover the embryo. After 10 min of fixation at room temperature, a second coverslip was overlaid onto the embryo which was consequently squeezed. Samples were frozen on dry ice and both coverslips were separated with a razor blade before a 3-min postfixation in −20°C methanol. Rehydration was done in PBS at room temperature. Embryos were subsequently treated for immunocytochemistry analysis as for extracts.

DNA synthesis in embryos was analyzed by 10 min of BrdU pulses, each for 5 min within a single cycle. Embryos were incubated for 10 min in 0.1× Barth's medium containing 4 mM BrdU and 0.5% DMSO. Embryos were taken between the 8- and 16-cell stages and between the 512- and

1,024-cell stages. They were first treated for 45 min in 0.5× PBS, 2 M HCl, and then further postfixed in methanol at −20°C.

Embryos or isolated nuclei on coverslips were incubated for 1 h at room temperature in PBS 2% BSA, 0.1% Tween 20 to block nonspecific interaction of the antibodies. The incubation with specific antibodies was overnight at 4°C in PBS, 2% BSA. After several washes in PBS, 0.1% Tween 20, the second FITC-conjugated or Texas red-conjugated streptavidin was added, following instructions from the manufacturers. To reveal biotin-dUTP, FITC or Texas red-conjugated streptavidin was mixed with the second antibody at the appropriate dilution. DNA was stained with either a 10-min incubation in 1 μg/ml Hoechst 33258 in PBS, or a 5-min incubation in 10^{−6} M propidium iodide in PBS. After several washes in PBS containing 0.1% Tween 20, slides were mounted in Citifluor antifading reagent (Canterbury, UK).

Microscopy and Image Analysis

Samples were examined using a BDS enhanced video microscope (Biological Detection Systems Inc., Pittsburg, PA). The system is based on a Zeiss Axiovert (Photometrics, Ltd., Tuscon, AZ) coupled to a charged-coupled device camera with low light level admission. Confocal microscopy was carried out using the TCS confocal imaging system (Leica Instruments, Heidelberg, Germany), equipped with a 63× objective (plan apo; NA = 1.4). For FITC and propidium iodide excitation, an argon-krypton ion laser adjusted to 488 and 568 nm was used. For each optical section, double fluorescence images were acquired in sequential mode (i.e., FITC first and propidium iodide second) The signal was treated with line averaging to integrate the signal collected over eight lines in order to reduce noise. The confocal pinhole was adjusted to allow a minimum depth of field. A focal

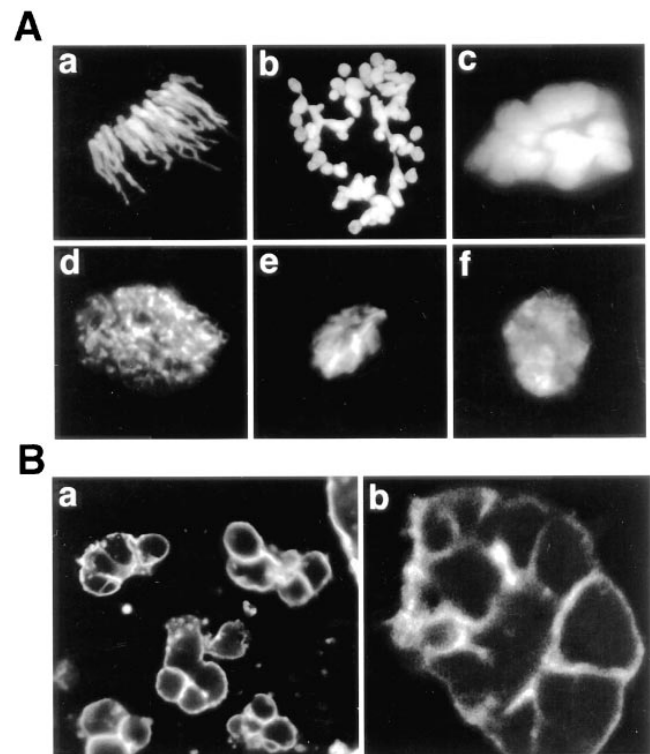


Figure 1. Nuclear reconstruction occurs from individualized chromosomes during early embryogenesis. (A) Changes in the reorganization of the chromatin is presented. DNA was analyzed by staining with Hoechst 33258 dye as described in Materials and Methods. Anaphase (a), telophase (b), interphase (c), and prophase (d) of nuclei before the 8,000-cell stage and telophase (e) and interphase (f) of nuclei after the 8,000-cell stage are shown. (B) Membranes associated with karyomeres (a) and newly formed nuclei (b) were detected by confocal microscopy using the lipid-staining dye DiOC6.

1. Abbreviations used in this paper: FB, fixation buffer; RPA, replication protein A.

series was collected for each specimen. The focus step between these sections was generally 0.5 μm . Focal series were then processed to produce single composite images (extended focus), combining high spatial resolution and high depth of field.

Results

Nuclear Dynamics and DNA Replication during the Early Embryonic Cell Cycles

When *Xenopus* embryos are analyzed during the early cleavage stage, specific nuclear structures are observed during each mitosis. During anaphase, each individual chromosome becomes surrounded by an envelope forming micronuclei-like structures distinctly observed at the beginning of telophase (Fig. 1 A, *a* and *b*). These individual vesicles grow and fuse during telophase (Fig. 1 A, *b* and *c*) to lead to more typical interphasic nuclei (Fig. 1, A *d*). The formation of the nuclear membrane around karyomeres and during the reconstruction of the nucleus has also been demonstrated by the membrane lipids dye DiOC6 (Fig. 1 B), indicating that during most of the cell cycle chromosomes behave as relatively independent micronuclei structures. Karyomeres are first detected at the two-cell-stage embryo, when the division furrow forms and represent the initial step of nuclear reconstruction during the entire segmentation period. At 8 h after fertilization, these structures disappear and nuclear reconstruction typical of somatic cells is observed (Fig. 1 A, *e* and *f* and Fig. 2). We conclude that karyomere formation is strictly specific to early development. This specific pathway was not observed later in embryonic development, nor in *Xenopus* somatic cells in culture. The abrupt disappearance of these structures after cycle 13 (Fig. 2) defines a novel transition occurring at blastula stage, which might reflect changes previously observed both in replication and transcriptional programs during development (21, 42).

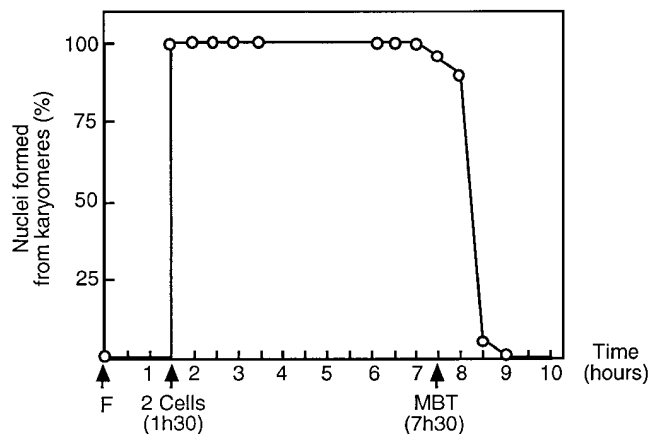


Figure 2. Karyomere formation is specific from the early embryogenesis. *Xenopus* embryos incubated at 21°C were taken from synchronized fertilized eggs at different times after fertilization. The kinetics and synchrony of cleavage were examined by light microscope during the first cell cycles; cycles were 30 min. The reorganization of the chromatin was analyzed by DNA staining with Hoescht 33258 dye as described in Materials and Methods. The plot shows the percentage of nuclei formed from karyomeres as a function of time after fertilization.

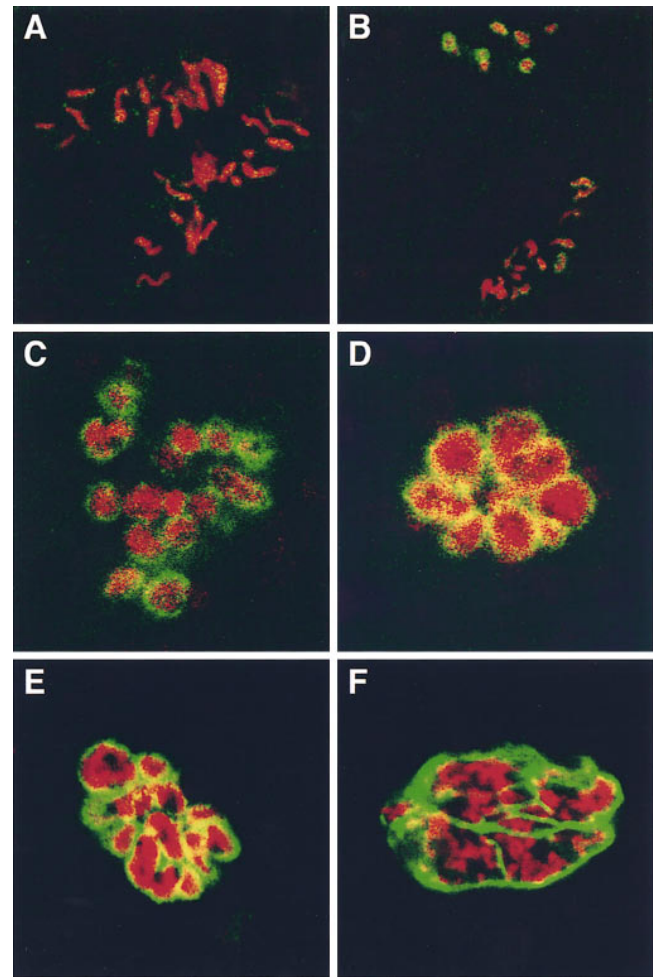


Figure 3. Lamins bind and surround each chromosome during the early embryonic cell cycles. Early embryos were prepared as described in Materials and Methods and submitted to indirect immunofluorescence visualization with a 687A7 monoclonal anti-lamin antibody (13). DNA was stained with propidium iodide. Green, lamins; red, DNA. A–F are successive stages of the organization of the nucleus during each cell cycle from anaphase (A) to prophase (F).

These specific features led us to consider to what extent the formation of karyomeres might explain the accelerated rate of DNA replication during early development. The formation of a nuclear envelope is essential to the initiation of DNA replication (4, 29, 40, 44) and also to prevent illegitimate reinitiation within the same cell cycle (4). In its absence, prereplication foci can form but DNA replication is not initiated (1). One essential component of the nuclear envelope that is required for the initiation of DNA replication is the lamina (16, 35, 44) and its presence or absence in karyomeres was not known. We used an antibody directed against the embryonic lamins to ask if they were incorporated into the karyomeres during the early cell cycles. Distinct small lamin spots were detected on mitotic chromosomes during anaphase (Fig. 3 A). The formation of the nuclear lamina proceeds asynchronously around each chromosome, with both punctuated and continuous layers of lamina on karyomeres migrating towards both

poles of the spindle (Fig. 3 *B*). Individual karyomeric vesicles grow and continuous lamina layers are observed around every karyomere (Fig. 3, *C* and *D*). Then the fusion of karyomeres occurs but internal folds remain detectable until late stages of nuclear reconstitution (Fig. 3, *E* and *F*). This pathway for lamina establishment was entirely specific for pre-midblastula transition (MBT) mitotic stages.

The observation of individual chromosomal vesicles surrounded by a lamina suggested that DNA replication might be underway before reconstruction of the nucleus and indeed before completion of mitosis. We used antibodies against three markers specific for different steps in DNA replication. The minichromosome maintenance protein XMCM3, a component of *Xenopus* licensing activity (6, 27, 34), and replication protein A (RPA) associate with sperm chromatin in egg extract (1, 49). XMCM3 was detected on migrating chromosomes during early anaphase (Fig. 4 *A*, *a* and *b*) and during nuclear reconstruction (data not shown). A similar staining was observed for other

members of the minichromosome maintenance (MCM) family of proteins (data not shown), identifying an early step in replication origin formation. RPA associates with prereplication centers, before the assembly of the nuclear envelope and the initiation of DNA replication (1, 49). In vivo, RPA was first detected during anaphase as a punctated staining on chromosomes (Fig. 4 *B*, *a* and *b*). When the nuclear lamina is formed around karyomeres, an intense and uniform RPA staining characteristic of ongoing DNA replication (1, 49) was observed (Fig. 4 *B*, *c* and *d*). Therefore, initiation of DNA replication starts in vivo at anaphase, and elongation begins in early telophase when chromosomes still behave as independent units. Synthesis of DNA was directly evaluated by incubating embryos with BrdU for 10-min pulses, and was detected in karyomeres during early telophase (Fig. 5 *A*). No incorporation of BrdU was observed in anaphase, confirming that DNA synthesis observed in karyomeres was not due to BrdU incorporation during the preceding cell cycle. Finally, proliferative cell nuclear antigen (PCNA), a processivity factor for DNA polymerase- δ was used as a specific marker for elongation of DNA synthesis (2, 5, 18), at a stage when the karyomeres are still distinct vesicles. PCNA was detected as a bright and non-punctated staining of karyomeres during early telophase, indicating that DNA synthesis was already occurring in karyomeres as the division furrow is just starting (Fig. 5 *B*, *a* and *b*). At this stage the mitotic spindle has not totally regressed (Fig. 5 *B*, *c* and *d*) and the blastomere has not yet divided. We conclude that DNA replication occurs in karyomeres while both mitosis and nuclear reconstruction are not yet completed.

An In Vitro System That Mimics DNA Replication and Mitotic Events Occurring in Karyomeres during the Embryonic Cell Cycles

Although karyomeres are observed as the initial step of nuclear reconstruction during the entire segmentation period, they were not observed in cell-free systems derived from *Xenopus* eggs, except in rare cases when a second cycle was observed (20). Nevertheless, *Xenopus* egg extracts are very efficient in DNA replication of demembrated sperm nuclei added in vitro (3, 19, 32). One hypothesis to explain this discrepancy could be that passage of prophase nuclei through mitosis is a crucial step in the formation of karyomeres during development.

To address this question, we used a novel *Xenopus* in vitro system prepared from early embryos at the 1,024-cell stage that were synchronized at the G2 phase by addition of cycloheximide. Such embryonic cells exhibit two centrosomes composed of two centrioles and pericentriolar material (14). The preparation of the extracts ensured that they already contained endogenous G2 nuclei. The addition of a mitotic extract containing MPF activity to this extract allowed the nuclei to progress synchronously into mitosis in vitro (Fig. 6 *A*, *a* and *b*). Nuclei arrested again at this stage, with a disassembled nuclear envelope and condensed chromosomes arranged in a metaphase plate with a typical bipolar spindle (Fig. 6 *A*, *b*). Completion of mitosis and progression to the following cell cycle then can be induced by addition of Ca^{2+} . Chromosomes segregate (Fig. 6 *A*, *c*), form karyomeres (Fig. 6 *A*, *d*), and then fuse

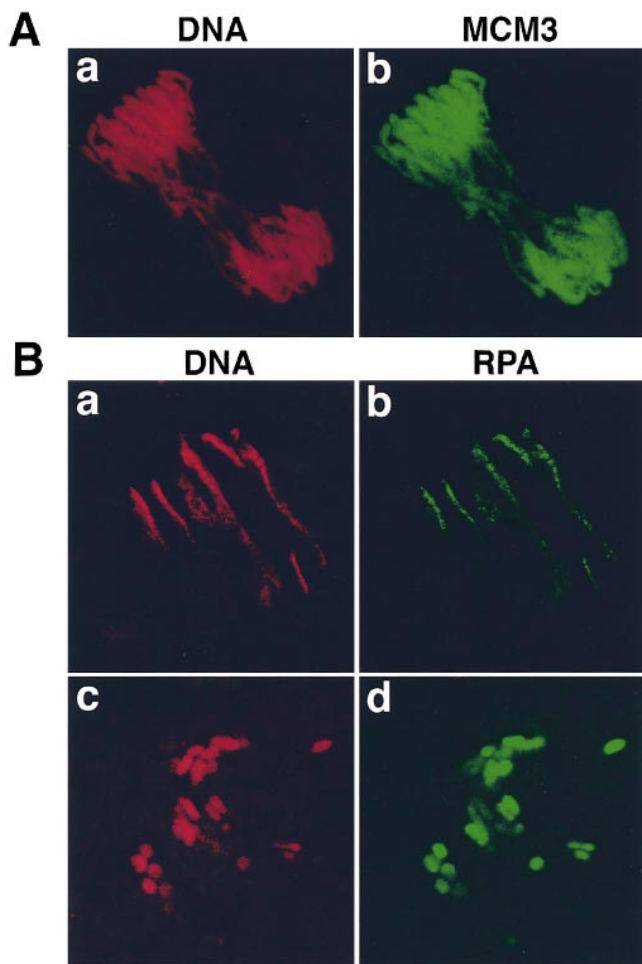


Figure 4. Prereplication centers are defined during anaphase. Early embryos were submitted to indirect immunofluorescence with XMCM3 (*A*) or RPA (*B*) antibodies. Red, DNA staining (propidium iodide); green, XMCM3 and RPA. Anaphase chromosomes at both poles (*A*, *a* and *b*) or one pole (*B*, *a* and *b*) and telophase karyomeres (*B*, *c* and *d*) are presented.

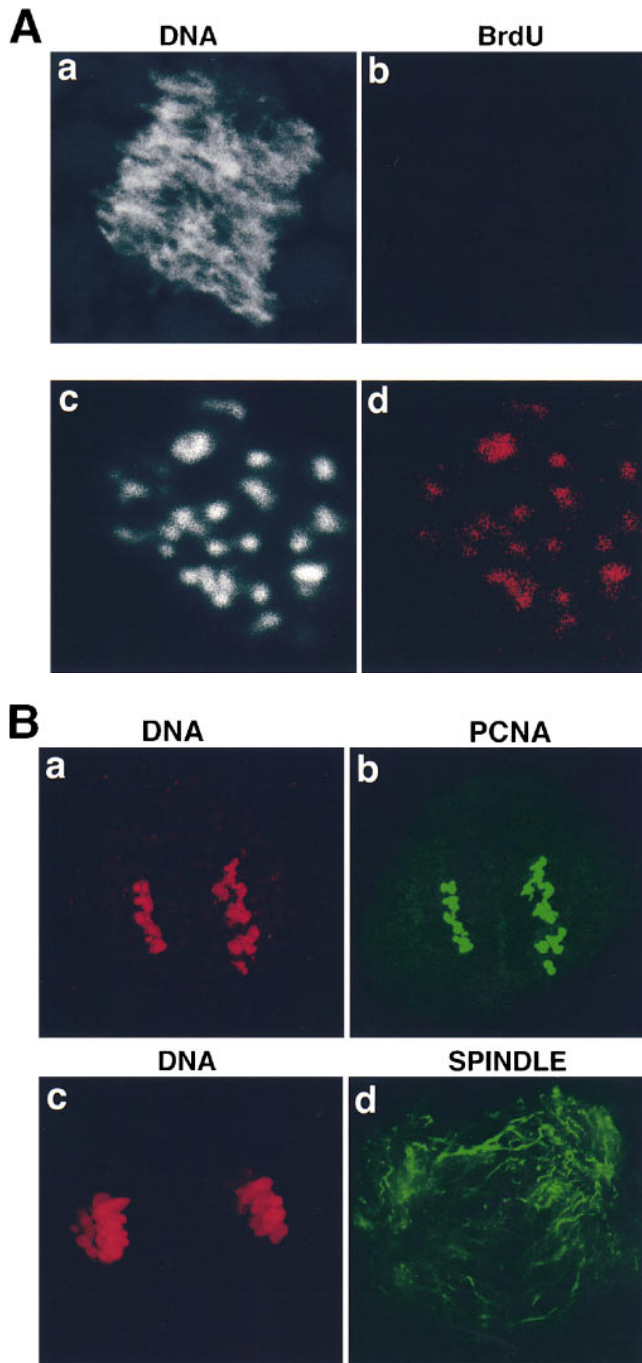


Figure 5. S phase occurs in karyomeres before nuclear formation and cell division in vivo. (A) Early embryos were incubated with BrdU for a 10-min pulse and prepared as described in Materials and Methods. BrdU incorporation was detected by indirect immunofluorescence after using an anti-BrdU antibody and DNA was visualized with Hoechst staining. BrdU incorporation is in red (b and d), and DNA in gray (a and c). Nuclei at anaphase (a and b) and early telophase (c and d) are shown. (B) Early embryos were examined by indirect immunofluorescence with PCNA and β tubulins antibodies. Green, PCNA (b) and β tubulins (d); red, DNA staining (propidium iodide) (a and c).

together to produce a G2-like nucleus 45–60 min after Ca^{2+} addition (Fig. 6 A, e and f). The extent of DNA replication, as measured by ^{32}P dATP incorporation with TCA precipitation, was consistent with one round of DNA synthesis and hence the completion of S phase (data not shown). We conclude that this acellular system faithfully mimics the successive steps of the organization of the nucleus observed in vivo during the early embryonic cell cycle, and that it is particularly suitable for the analysis of the transition between mitosis and the onset of the S phase. When biotin-dUTP was added to the extract, DNA replication was first detected during telophase, starting asynchronously in karyomeric vesicles before nuclear reconstruction (Fig. 6 B), consistent with the asynchrony in the establishment of the lamina around individual karyomeres (refer to Fig. 3 B). Initiation of DNA replication starts on karyomeres whereas chromatin is not fully decondensed and presents a punctated RPA staining (Fig. 6 B). Other karyomeres display decondensed chromatin and homogeneous biotin-dUTP and RPA staining characteristic of later stages of replication (Fig. 6 B). Karyomeres can be at different stages of replication when they fuse, indicating that during early development the unit of replication is not the nucleus but the chromosome.

Karyomere Formation and Fusion Does Not Depend on DNA Synthesis but Requires Chromosome Segregation

When aphidicolin is added to the extract, DNA synthesis is blocked but nuclear reconstruction from segregated karyomeres nevertheless occurs (Fig. 7, C and D). The same result was observed in vivo in early embryos in the presence of aphidicolin (data not shown). We conclude that during early development, karyomere formation and reconstitution of the nucleus from karyomeres are not directly linked to the DNA replication process.

Nocadazole added to the extract prevents spindle formation and chromosome segregation after dissolution of the nuclear membrane. In these conditions, karyomeres were not formed but a nuclear envelope develops around the chromosomes (Fig. 7, E and F), as during telophase in post-MBT embryos or in somatic cells. We conclude that karyomere formation requires the segregation of chromosomes at each mitosis. This explains the observation that sperm nuclei do not form karyomeres in an interphasic extract, as chromosomes do not segregate in this context.

Discussion

A Specific Nuclear Structure Adapted to the Rapid Embryonic Cell Cycles

The formation of karyomeres during early development is probably permitted by the maternal accumulation of proteins and membrane components required to assemble functional nuclear envelopes for $\sim 4,000$ nuclei (31, 32, 43, 48). The mitotic spindle is also essential for the formation of karyomeres. First, it permits chromosomes to behave separately; if the formation of the mitotic spindle is prevented, the nucleus reforms around the entire mass of chromosomes. Second, the size of the egg (1.2 mm) imposes long mitotic spindles during the first cleavages of the

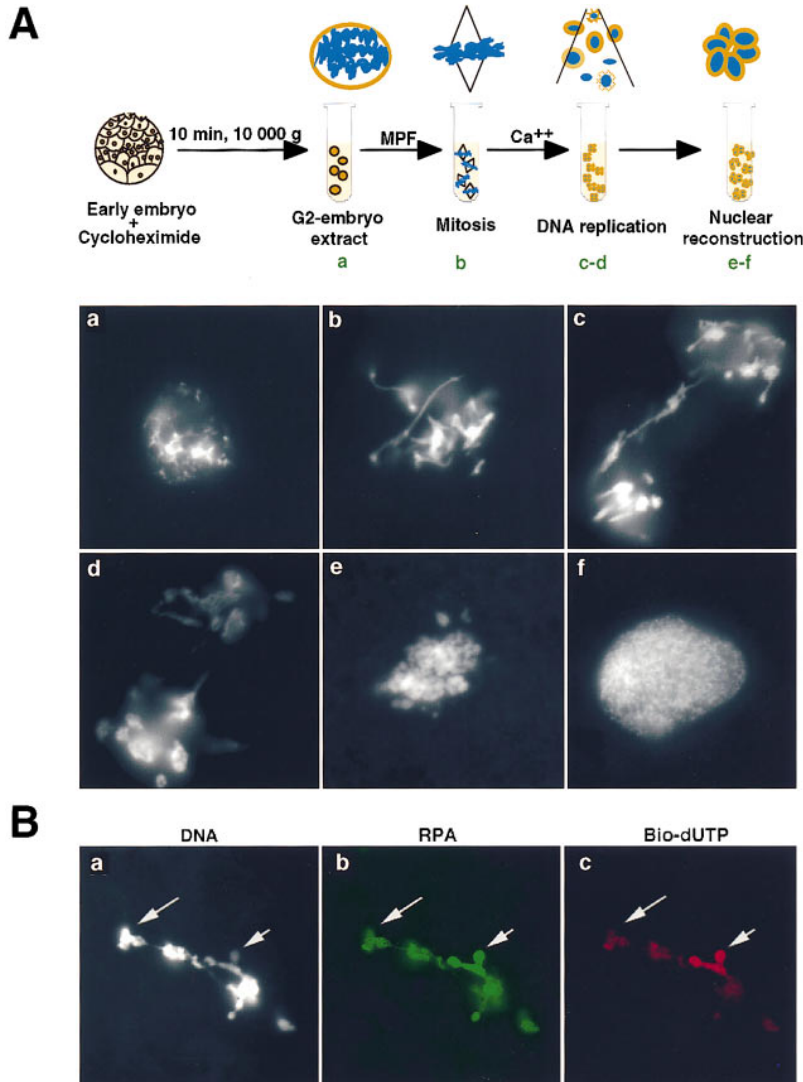


Figure 6. (A) An embryonic extract which mimics the early embryonic cell cycles. An extract was prepared from early embryos containing G2 synchronized nuclei, as described in Materials and Methods. The extracts were incubated for 30 min at 23°C after addition of a metaphase-arrested egg extract. Under these conditions, the nuclear membrane breaks down and nuclei arrest at the metaphase stage of mitosis. Completion of mitosis and S phase was induced by addition of 0.6 mM CaCl₂. Samples were then placed onto slides and fixed as described as in Materials and Methods. (a) G2/prophase embryo nucleus; (b) metaphase; (c) anaphase, 15 min after Ca⁺⁺ activation; (d) early telophase, 15–30 min after Ca⁺⁺ activation; (e) mid-telophase 30 min after Ca⁺⁺ activation; (f) prophase 45–60 min after Ca⁺⁺ activation. DNA was stained by Hoechst 33258 and observed by video enhanced microscopy. (B) DNA replication occurs in karyomeres in vitro. An extract from embryos synchronized in G2 was induced to undergo mitosis by addition of MPF activity as described in Materials and Methods. Biotin-dUTP (20 μM) was then added and completion of mitosis and S phase was induced by further addition of 0.6 μM CaCl₂. Samples were analyzed for biotin-dUTP incorporation 15–30 min after activation by Ca²⁺. Biotin in DNA was detected by indirect immunofluorescence with streptavidin Texas red (c) and RPA (b) immunostaining was detected with specific antibody. DNA was stained with Hoescht 33258 dye (a). Long and short arrows show condensed and decondensed chromatin respectively into independent karyomeres, respectively.

embryo, and only by the blastula stage does the size of the cells approach that of a somatic cell. Assuming that the average rate of chromosome movement is constant (39), the regroupment of the chromosomes at the poles will be more rapid at the end of the cleavage stage. Moreover, membrane components also become limiting at the blastula stage. Therefore, the disappearance of karyomeres at this stage may be a consequence of both the depletion of maternal components for nuclei construction and the uncoupling between growth and division during early embryogenesis. Although karyomere formation is a physiological strategy developed by the embryo, the structure of karyomeres is reminiscent of micronuclei. Micronuclei formation in normal somatic cells can occur as a consequence of a variety of physical or chemical damaging agents (8–10, 30, 45). Most physical or chemical reagents that damage the mitotic spindle or inhibit its formation produce micronuclei due to failure to properly assemble chromosomes at the mitotic poles. Disruption of the function of NuMA, a protein involved in the architecture of the mitotic spindle, also results in the formation of micronuclei (24, 25). The

size of the cells in the early embryo that imposes a long transit time to the spindle poles may parallel the situation in somatic cells when the mitotic spindle is damaged, leading to a disturbed migration of chromosomes and micronuclei formation.

Thus, we think that the organization in karyomeres during early development occurs as a consequence of the timing for segregation and reassembly of chromosomes at the spindle poles relative to the acceleration of other cell cycle events permitted by previously stored maternal components.

Chromosomes as Independent Units of DNA Replication

Our observations both in vivo and in vitro reveal an organization for DNA replication during early development which is relatively simpler than in somatic cells (Fig. 8). DNA replication is initiated and proceeds within karyomeres before nuclear reconstruction. Prereplicating complexes, as detected by RPA immunolocalization, form on

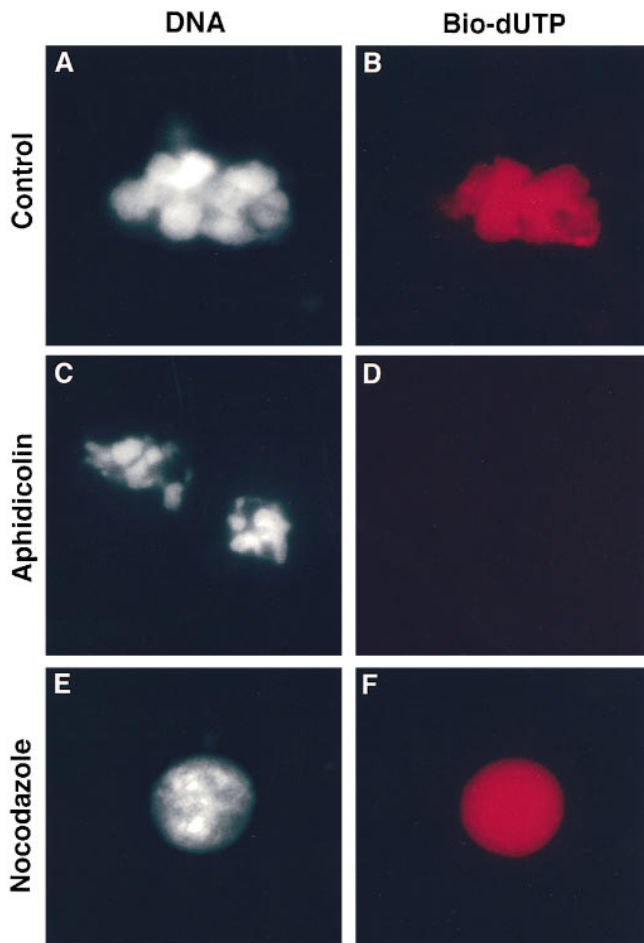


Figure 7. Karyomere formation occurs independently of DNA replication but requires spindle formation. An embryonic extract containing endogenous G2 nuclei was released into mitotic metaphase as in Materials and Methods (A and B). DNA replication was blocked by 50 $\mu\text{g/ml}$ aphidicolin (C and D), and spindle formation was prevented by 5 $\mu\text{g/ml}$ nocodazole (E and F). Biotin-dUTP (20 μM) was added and progression into S phase was induced by 0.6 mM CaCl_2 . DNA synthesis was followed by biotin-dUTP incorporation after 15–30 min of incubation in B, D, and F. DNA was stained by Hoescht 33258 (A, C, and E).

condensed chromosomes in anaphase and DNA synthesis initiates in karyomeres at the beginning of telophase. This behavior is in accordance with the licensing-loading factor hypothesis which postulates that licensing DNA for replication occurs during mitosis before nuclear envelope formation (4). Formation of the nuclear lamina that is essential to the initiation of DNA replication (16, 23, 36) occurs within karyomeres during anaphase–telophase.

As opposed to the context in somatic cells, where some factors specific for S phase are produced only in G1, all components necessary for DNA replication are already present in the unfertilized egg. Therefore, the isolation of chromosomes into independent karyomeric vesicles allows both the formation of prereplication centers on condensed chromosomes during anaphase and the initiation of DNA synthesis before nuclear formation. A quite unusual situation results, in which a 2n phase nucleus is virtually absent

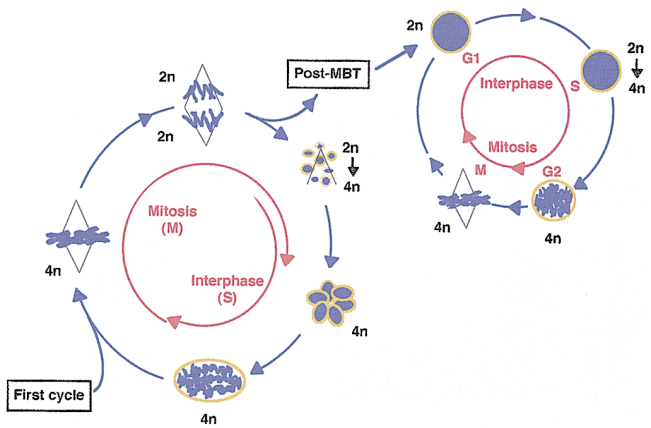


Figure 8. The cell cycle during early development and after MBT. At each cell cycle during the first twelve divisions, chromosomes, during their segregation to the poles, form karyomeres wherein DNA replication begins. A distinct transition occurs at the mid-blastula stage when karyomeres are no longer formed during segregation. The nucleus is then reconstructed from 2n chromosomes, and a classical cell cycle appears for the first time during development.

during the entire early embryonic development (Fig. 8). This phenomenon might also account for a peculiarity of the genus *Xenopus*, which is that all but one of its 20 species are of ancient polyploid origin. Moreover, they form a polyploid series in the proportion 2:4:8:12 (26). The partial overlap between S and M phase together with the early formation of a nuclear envelope during the first embryonic divisions might have contributed to a relative facility to polyploidization.

This specific organization, where the unit of replication is not the nucleus but the chromosome, might also favor a higher frequency of initiation of DNA replication. Prereplication complexes studied by confocal microscopy of RPA foci in early embryonic nuclei were smaller and two- to threefold more numerous when nuclei were formed from karyomeres (unpublished observations). Moreover, the increase in the number of prereplication centers also correlates with a decreased size of the DNA loop domains. Using the maximum fluorescent halo technique (47), we measured a mean DNA loop size in karyomeres of 14.6 kb (data not shown) similar to the mean replicon size during early *Xenopus* development (22). This structure may provide a structural framework for an accelerated rate of replication during the early developmental period.

The abrupt disappearance of karyomeres after the twelfth cell cycle is a new transition occurring at the mid-blastula stage, which might affect not only replication but also transcriptional programs during development. During early development, a cell cycle clock, driven by the cycling MPF activity, imposes cell division every 30 min whether or not DNA is replicated (17, 41). The organization of the genome in karyomeres appears to be a developmental strategy to permit both a reduced replication period and an early initiation, so enabling replication to keep pace with the cytoplasmic clock. The formation of a nuclear envelope around isolated chromosomes also provides an

vivo validation of its role in regulating initiation of DNA replication (4). Indeed, as soon as a nuclear envelope isolates chromosomes, they can replicate once and only once, even if initiation of the whole set of chromosomes is permitted before the end of the cell cycle.

We are grateful to U.K. Laemmli (University of Geneva, Science II, Geneva, Switzerland) for the RPA antibody, R.A. Laskey (Wellcome CRC Institute, Cambridge, UK) for the anti-MCM3 antibody, and R. Stick (University of Göttingen, Göttingen, Germany) for the lamin antibody. We also thank P. Brooks (Genoscope, Evry, France), J.C. Courvalin, and T. Grange (both from Institut Jacques Monod, Paris, France) for critical reading of this manuscript, and the photographic department of the Institut Jacques Monod.

This study has been supported by grants from the CNRS, Association pour la Recherche sur le Cancer, the Ligue Nationale contre le Cancer, the Fondation de la Recherche Médicale, and the Groupements des entreprises dans la lutte contre le cancer.

Received for publication 18 May 1998 and in revised form 28 July 1998.

References

- Adchi, Y., and U.K. Laemmli. 1994. Study of the cell cycle-dependent assembly of the DNA pre-replication centres in *Xenopus* egg extracts. *EMBO (Eur. Mol. Biol. Organ.) J.* 13:4153–4164.
- Baker, T.A., and S.P. Bell. 1998. Polymerases and the replisome: machines within machines. *Cell.* 92:295–305.
- Blow, J.J., and R.A. Laskey. 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 R.A. Laskey. 1988. A role for the nuclear envelope in controlling DNA replication within the cell cycle. *Nature.* 332:546–548.
- Bravo, R., R. Frank, P.A. Blundell, and H. Macdonald-Bravo. 1987. Cyclin/PCNA is the auxiliary protein of DNA polymerase-delta. *Nature.* 326:515–517.
- Chong, J.P., H.M. Mahbubani, C.Y. Khoo, and J.J. Blow. 1995. Purification of an MCM-containing complex as a component of the DNA replication licensing system. *Nature.* 375:418–421.
- Coue, M., S.E. Kearsey, and M. Mechali. 1996. Chromatin binding, nuclear localization and phosphorylation of *Xenopus* cdc21 are cell-cycle dependent and associated with the control of initiation of DNA replication. *EMBO (Eur. Mol. Biol. Organ.) J.* 15:1085–1097.
- Crenshaw, A.H., Jr., J.W. Shay, and L.R. Murrell. 1981. Colcemid-induced micronucleation in cultured human cells. *J. Ultrastruct. Res.* 75:179–186.
- Ege, T., H. Hamberg, U. Kron Dahl, J. Ericsson, and N.R. Ringertz. 1974. Characterization of minicells (nuclei) obtained by cytochalasin enucleation. *Exp. Cell Res.* 87:365–377.
- Ege, T., N.R. Ringertz, H. Hamberg, and E. Sidebottom. 1977. Preparation of microcells. *Methods Cell Biol.* 15:339–357.
- Elledge, S.J. 1996. Cell cycle checkpoints: preventing an identity crisis. *Science.* 274:1664–1672.
- Emanuelsson, H. 1973. Karyomeres in early cleavage embryos of *Ophrotrocha labronica* LaGreca and Bacci. *Wilhelm Roux' Archiv.* 173:27–45.
- Firmbach-Kraft, I., and R. Stick. 1995. Analysis of nuclear lamin isoprenylation in *Xenopus* oocytes: isoprenylation of lamin B3 precedes its uptake into the nucleus. *J. Cell Biol.* 129:17–24.
- Gard, D.L., S. Hafezi, T. Zhang, and S.J. Doxsey. 1990. Centrosome duplication continues in cycloheximide-treated *Xenopus* blastulae in the absence of a detectable cell cycle. *J. Cell Biol.* 110:2033–2042.
- Gerhart, J., M. Wu, and M. Kirschner. 1984. Cell cycle dynamics of an M-phase-specific cytoplasmic factor in *Xenopus laevis* oocytes and eggs. *J. Cell Biol.* 98:1247–1255.
- Goldberg, M., H. Jenkins, T. Allen, W.G. Whitfield, and C.J. Hutchison. 1995. *Xenopus* lamin B3 has a direct role in the assembly of a replication competent nucleus: evidence from cell-free egg extracts. *J. Cell Sci.* 108:3451–3461.
- Hara, K., P. Tydeman, and M. Kirschner. 1980. A cytoplasmic clock with the same period as the division cycle in *Xenopus* eggs. *Proc. Natl. Acad. Sci. USA.* 77:462–466.
- Hutchison, C., and I. Kill. 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., R. Cox, R.S. Drepaull, M. Gomperts, and C.C. Ford. 1987. Periodic DNA synthesis in cell-free extracts of *Xenopus* eggs. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:2003–2010.
- Hutchison, C.J., R. Cox, and C.C. Ford. 1988. The control of DNA replication in a cell-free extract that recapitulates a basic cell cycle in vitro. *Development (Camb.)*. 103:553–566.
- Hyrien, O., C. Maric, and M. Mechali. 1995. Transition in specification of embryonic metazoan DNA replication origins. *Science.* 270:994–997.
- Hyrien, O., and M. Mechali. 1993. Chromosomal replication initiates and terminates at random sequences but at regular intervals in the ribosomal DNA of *Xenopus* early embryos. *EMBO (Eur. Mol. Biol. Organ.) J.* 12:4511–4520.
- Jenkins, H., W.G. Whitfield, M.W. Goldberg, T.D. Allen, and C.J. Hutchison. 1995. Evidence for the direct involvement of lamins in the assembly of a replication competent nucleus. *Acta Biochim. Pol.* 42:133–143.
- Kallajoki, M., J. Harborth, K. Weber, and M. Osborn. 1993. Microinjection of a monoclonal antibody against SPN antigen, now identified by peptide sequences as the NuMA protein, induces micronuclei in PtK2 cells. *J. Cell Sci.* 104:139–150.
- Kallajoki, M., K. Weber, and M. Osborn. 1991. A 210 kDa nuclear matrix protein is a functional part of the mitotic spindle; a microinjection study using SPN monoclonal antibodies. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:3351–3362.
- Kobel, H.R., and L. Du Pasquier. 1986. Genetics of polyploid *Xenopus*. *Trends Genet.* 2:310–315.
- Kubota, Y., S. Mimura, S. Nishimoto, H. Takisawa, and H. Nojima. 1995. Identification of the yeast MCM3-related protein as a component of *Xenopus* DNA replication licensing factor. *Cell.* 81:601–609.
- Lemaitre, J.M., S. Bocquet, R. Buckle, and M. Mechali. 1995. Selective and rapid nuclear translocation of a c-Myc-containing complex after fertilization of *Xenopus laevis* eggs. *Mol. Cell Biol.* 15:5054–5062.
- Leno, G.H., and R.A. Laskey. 1991. The nuclear membrane determines the timing of DNA replication in *Xenopus* egg extracts. *J. Cell Biol.* 112:557–566.
- Lewis, W.H. 1947. Interphase (resting) nuclei, chromosomal vesicles and amitosis. *Anat. Rec.* 97:433–445.
- Lohka, M.J., and J.L. Maller. 1985. Induction of nuclear envelope breakdown, chromosome condensation, and spindle formation in cell-free extracts. *J. Cell Biol.* 101:518–523.
- Lohka, M.J., and Y. Masui. 1983. Formation in vitro of sperm pronuclei and mitotic chromosomes induced by amphibian ooplasmic components. *Science.* 220:719–721.
- Longo, F.J. 1972. An ultrastructural analysis of mitosis and cytokinesis in the zygote of the sea urchin, *Arbacia punctulata*. *J. Morphol.* 138:207–238.
- Madine, M.A., C.Y. Khoo, A.D. Mills, and R.A. Laskey. 1995. MCM3 complex required for cell cycle regulation of DNA replication in vertebrate cells. *Nature.* 375:421–424.
- Meier, J., K.H. Campbell, C.C. Ford, R. Stick, and C.J. Hutchison. 1991. The role of lamin LIII in nuclear assembly and DNA replication, in cell-free extracts of *Xenopus* eggs. *J. Cell Sci.* 98:271–279.
- Moir, R.D., M. Montag-Lowy, and R.D. Goldman. 1994. Dynamic properties of nuclear lamins: lamin B is associated with sites of DNA replication. *J. Cell Biol.* 125:1201–2012.
- Montag, M., H. Spring, and M.F. Trendelenburg. 1988. Structural analysis of the mitotic cycle in pre-gastrula *Xenopus* embryos. *Chromosoma.* 96:187–196.
- Murray, A.W. 1991. Cell cycle extracts. *Methods Cell Biol.* 36:581–605.
- Murray, A.W., A.B. Desai, and E.D. Salmon. 1996. Real time observation of anaphase in vitro. *Proc. Natl. Acad. Sci. USA.* 93:12327–12332.
- Newport, J. 1987. Nuclear reconstitution in vitro: stages of assembly around protein-free DNA. *Cell.* 48:205–217.
- Newport, J., and M. Dasso. 1989. On the coupling between DNA replication and mitosis. *J. Cell Sci.* 12(Suppl.):149–160.
- Newport, J., and M. Kirschner. 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.W., and D.J. Forbes. 1987. The nucleus: structure, function, and dynamics. *Annu. Rev. Biochem.* 56:535–565.
- Newport, J.W., K.L. Wilson, and W.G. Dunphy. 1990. A lamin-independent pathway for nuclear envelope assembly. *J. Cell Biol.* 111:2247–2259.
- Shay, J.W., and M.A. Clark. 1977. Morphological studies on the enucleation of colchicine-treated L-929 cells. *J. Ultrastruct. Res.* 58:155–161.
- Sherr, C.J. 1996. Cancer cell cycles. *Science.* 274:1672–1677.
- Vogelstein, B., D.M. Pardoll, and D.S. Coffey. 1980. Supercoiled loops and eucaryotic DNA replication. *Cell.* 22:79–85.
- Wilson, K.L., and J. Newport. 1988. A trypsin-sensitive receptor on membrane vesicles is required for nuclear envelope formation in vitro. *J. Cell Biol.* 107:57–68.
- Yan, H., and J. Newport. 1995. An analysis of the regulation of DNA synthesis by cdk2, Cip1, and licensing factor. *J. Cell Biol.* 129:1–15.