Spontaneous Formation of Nucleus-Like Structures around Bacteriophage DNA Microinjected into Xenopus Eggs

Douglass J. Forbes, Marc W. Kirschner, and John W. Newport
Department of Biochemistry and Biophysics
School of Medicine
University of California
San Francisco, California 94143

Summary

We have found that injection of bacteriophage $\lambda$ DNA into unfertilized Xenopus eggs causes the assembly around the DNA of structures resembling typical eucaryotic cell nuclei. These spherical structures begin to form 60–90 min after injection. They contain $\lambda$ DNA and are bounded by a phase-dense envelope. Immunofluorescent staining of the $\lambda$-DNA-containing structures with anti-lamin antibody reveals the presence of the lamin nuclear proteins at the periphery of the structure, a pattern identical to that of embryonic nuclei. Electron microscopy reveals that the injected DNA is surrounded by a double bilayer nuclear membrane containing nuclear pore complexes. The “nuclei” containing $\lambda$ DNA respond to modulators of the Xenopus cell cycle in a manner that mimics the response of embryonic nuclei to these modulators during mitosis. These results suggest that nuclear reassembly and breakdown occur independently of specific DNA sequence information.

Introduction

The definitive characteristic of a eucaryotic cell is the segregation of genomic DNA into a separate organelle, the nucleus. The structure of the nucleus has been investigated by many groups (see Busch, 1973, for review) and a composite picture has begun to emerge. The outermost structure of the nucleus is the nuclear envelope, composed of an inner and outer membrane interrupted by frequent nuclear pore complexes (Aaronson and Blobel, 1975; for reviews see Kay and Johnston, 1973; Franke and Scheer, 1974; Fry, 1976). Through these pores macromolecules are transported. Lining the inside of the envelope is the nuclear lamina, a proteinaceous layer composed of one or more of the lamin proteins A, B, C (Gerace and Blobel, 1980; Krohne et al., 1981). Within the nucleus are the chromatin, macroscopically arranged as chromosomes, the nucleolus, and the nuclear matrix (Busch and Smetana, 1970; Berezney and Coffey, 1977; Berezney and Buchholtz, 1981; Hunt and Vogelstein, 1981; Maul, 1982). Each of these nuclear structures must be disassembled and reassembled every cell cycle.

During the early developmental stages of both Xenopus and Drosophila, cell division occurs every 10–30 min. Thus the complex nuclear structure in these organisms during this period must be temporally very plastic (Zalokar, 1976; McKnight and Miller, 1976; Satow, 1977; Newport and Kirschner, 1982a; Foe and Alberts, 1983). Studies of early embryos during this rapid cleavage period have shown that the nuclear structure reassembles in a two-stage process, the first step of which is the formation of nuclear envelopes around individual mitotic (telophase) chromosomes to form “mini-nuclei” or karyomers. These karyomers then fuse together to form a single interphase nucleus (Wilson, 1900; Harris, 1961; Ito et al., 1981). The individual chromosome vesicles enter S phase of the cell cycle, replicating their DNA even before fusion. Thus it is clear from these studies that a single chromosome can “seed” formation of a mini-nucleus, resembling the full nucleus both in structure and ability to enter S phase of the cell cycle. Presumably, at the end of mitosis, there is a cellular signal that initiates reassembly of the nuclear structure, while at the end of interphase, another cellular signal initiates the breakdown of the nuclear envelope in preparation for mitosis.

The frog egg has been shown to be a unique cell in which a number of common cellular components are stored for later in development. The egg contains a large pool of histones sufficient for 20,000 cells (Woodland and Adamson, 1977; Laskey et al., 1977). If DNA is injected into the egg it is assembled into apparently native chromatin (Laskey et al., 1977; Newport and Kirschner, 1982a; Hartland et al., 1983). When demembranated sperm nuclei are added to a cytoplasmic extract of activated eggs, the nuclei reacquire a nuclear envelope, indicating the presence of a pool of envelope components in the egg cytoplasm (Lohka and Masui, 1983). The presence of large pools of nuclear RNA-binding proteins and small nuclear RNAs in the egg has also been shown, and reconstitution of snRNP particles is possible upon injection of exogenous snRNA (De Robertis et al., 1982; Zeller et al., 1983; Forbes et al., 1983). DNA polymerase, tubulin, and ribosomes are stored in the egg in equally large amounts (Benbow and Ford, 1975; Pestell, 1975; Woodland and Adamson, 1977). Because the cell cycle in the early Xenopus embryo is only 30 min, it is reasonable to assume that most of the other structural components of the nucleus are also stored in large concentrations. In this report we show that this appears to be true.

We find that when bacteriophage $\lambda$ DNA is injected into Xenopus eggs, this DNA is assembled into nucleus-like structures containing a nuclear envelope, double nuclear membrane, and nuclear pores. Furthermore, these structures respond to modulators of the cell cycle in a manner identical to that of embryonic nuclei. These results strongly suggest that: 1) nuclear assembly is a process that occurs on a DNA template, and specific DNA sequences are not necessary for this process to occur; 2) certain modulators of the cell cycle do not require the presence of specific nuclear DNA sequences in order to act.
Results

Nucleus-Like Structures Form around DNA Injected into Xenopus Eggs

When linear double-stranded bacteriophage λ DNA was injected into unfertilized eggs it initially appeared as a dispersed fibrous network, as visualized by fluorescence microscopy. An example of such a network is shown in Figure 1A. In this experiment, an egg was injected with λ DNA (5–8 ng/50 nl) and immediately lysed and stained with the polynucleotide-specific fluorescent dye, bisbenzimide (Hoechst-33258). The injected DNA was located using a fluorescence microscope. If the injected DNA was allowed to incubate in the egg for 90 min before lysis, it was found that it had now acquired a large degree of structural organization (Figure 1B).

The structural reorganization of the injected DNA appeared to occur in three steps. First, the DNA filaments, seen in Figure 1A, became shorter and thicker in a process that took approximately 30–45 min. This initial condensation process may reflect the conversion of naked DNA into chromatin, a process that is known to occur rapidly and efficiently in Xenopus eggs (Laskey et al., 1977; Newport and Kirschner, 1982b). The initial condensation was followed by a period in which the DNA began to aggregate into a densely packed spherical configuration (Figure 1B). By 90 min after injection into the egg these densely packed DNA-containing structures began to "bud off" from the larger mass of DNA (Figure 1B). At this point, the DNA appeared to be surrounded by a membranous envelope, as seen by phase contrast microscopy (Figure 2A). Such envelopes continue to increase in surface area and, as a consequence of this swelling, the enclosed DNA becomes less densely packed. Single spherical structures varied in size from 1 to 20 μm in diameter, depending upon the amount of DNA enclosed. If these structures were optically sectioned by adjusting the focal plane of the microscope through them, the bulk of the DNA appeared to be bound

Figure 1. Fate of Bacteriophage DNA Injected into Xenopus Eggs

Bacteriophage λ DNA (5–8 ng) was injected into unfertilized Xenopus eggs. The injected eggs were lysed at different times in the Hoechst dye, bisbenzimide, and 3.7% formaldehyde by the addition of a cover slip, as described in Experimental Procedures. The structure of the injected DNA was observed by fluorescence microscopy (about 220-fold magnification). (A) DNA in an egg lysed immediately after injection, showing the DNA as a disperse fibrous array; (B) DNA in an egg lysed 90 min after injection. The three stages of DNA rearrangement are shown: 1) partially condensed DNA, 2) highly condensed and partially spherical DNA, and 3) budding spherical nucleus-like structures.
either directly to or in close proximity to the surrounding envelope, based on fluorescence intensities (not shown). Using phase contrast optics, it could be seen that the \( \lambda \) DNA-containing structures were bounded by a well-defined envelope that excluded the surrounding yolk granules (Figure 2A). Figure 2B shows the bisbenzimide staining of the DNA in the same nucleus at a slightly higher magnification. The envelope of such nuclei is resistant to exposure to 0.2% Triton-X100, suggesting that it is not exclusively phospholipid in composition.

These same nucleus-like structures assembled around both linear bacteriophage T4 DNA and circular plasmid pBR322 DNA when the DNAs were injected into the egg. Induction of nuclear structures around injected DNA appears to be independent of both specific DNA sequences and linear arrangement of the DNA. It should be noted, however, that the structures formed around the pBR322 DNA were much smaller and much more fragile than those that formed around either \( \lambda \) or T4 DNA.

The assembly of injected \( \lambda \) DNA into a well-defined, envelope-enclosed structure suggests that the DNA is being organized into a nucleus-like structure. For this reason and for convenience we have chosen to refer to these spontaneously induced structures as "nuclei" in this report. Naturally occurring nuclei will be referred to as embryonic nuclei, somatic nuclei, or endogenous nuclei to distinguish them from nuclei containing \( \lambda \) DNA.

In order to demonstrate that the nuclei we see via fluorescence are indeed derived from the injected \( \lambda \) DNA and not from replication of the endogenous maternal nucleus, two types of experiments were done. First, eggs were injected with buffer, lysed 90 min later in bisbenzimide dye and fixative, and examined with the fluorescence microscope. At most one nucleus was seen (presumably maternally derived), whereas when \( \lambda \) DNA was injected (6 ng/egg) 50–200 nuclei were seen. Second, the maternal nucleus can be manually removed from an egg by removing cytoplasm from just under the cortex in the animal hemisphere of the egg. That the maternal nucleus has been removed can be confirmed by staining the withdrawn cytoplasm for DNA with bisbenzimide dye. When we removed the maternal nucleus and then injected \( \lambda \) DNA, nuclei formed in identical number and with the same time course as was found in eggs containing the maternal nucleus. Thus we conclude that the presence of the maternal nucleus does not play a role in the formation of nuclei derived from injected \( \lambda \) DNA.

To demonstrate that it is the bacteriophage DNA that is contained within these nuclei, \( \lambda \) DNA was labeled with \(^{3}H\)-dCTP in vitro and injected into the egg. After 90 min, these eggs were fixed, embedded in paraffin, sectioned, and autoradiographed. As can be seen in Figure 2B, the majority of silver grains are contained within the newly formed nuclei. This demonstrates that it is \( \lambda \) DNA that is present in the nuclei and that most of the \( \lambda \) DNA is found in these structures.

**Nuclei Spontaneously Formed around Injected DNA Contain Nuclear Lamins, Membranes, and Structures Typical of Nuclear Pores**

The major structural proteins of the nuclear envelope are the nuclear lamins. Using a highly specific, high-titer antibody against the nuclear lamin proteins, which was derived from a human patient with the autoimmune disease scleroderma (McKeon et al., 1983), the \( \lambda \)-DNA-induced nuclei were examined for the presence of nuclear lamins. Eggs were injected with \( \lambda \) DNA, incubated 90 min, fixed, and sectioned. The frozen sections were stained first with anti-lamin antisera and then with rhodamine-labeled anti-human IgG antisera. The \( \lambda \)-DNA-containing nuclei were found to stain with anti-lamin antisera, having a brightly fluorescent outer perimeter enclosing a central core of DNA (Figures
4A, B, the DNA staining, visualized with bisbenzimide dye, is not shown). This pattern is identical to the staining pattern found when endogenous Xenopus embryonic nuclei were stained with the anti-lamin antisera (Figure 4C). This result demonstrates that the nuclear envelope surrounding these \( \lambda \)-DNA-containing nuclei is composed of the same major structural lamin proteins present in normal embryonic nuclei.

Electron micrographs of eggs injected with \( \lambda \) DNA and allowed to incubate for 2 hr before fixation and sectioning revealed vesicles of the same number and size as seen by fluorescence microscopy, as described above. These vesicles contained a double membrane resembling both the inner and outer nuclear membrane (Figure 5A). On one side of the nuclear envelope cytoplasmic structures such as mitochondria and yolk granules were found, while on the other side osmiophilic material of the same density and staining as interphase chromatin was seen. The nuclear membranes appeared to be periodically interrupted by typical nuclear pores (Figures 5B, 5C). The nuclear envelope and the pores contained within it are identical in structure to the nuclear envelope characteristic of pre-midblastula embryonic nuclei (Figure 5D). The pores appear to be spaced about the same distance apart in both embryonic and \( \lambda \)-DNA-containing nuclei.

Response of Spontaneously Formed Nuclei to Cell Cycle Regulation
The early embryonic cell cycle in Xenopus is biphasic, consisting of only S phase (DNA replication) and M phase (mitosis), without the usual G1 and G2 phases seen in most somatic cells (Graham and Morgan, 1966). Either fertilization or pricking the egg with a needle will activate the egg to begin an oscillation between S and M phases of the cell cycle. This cycling occurs even in the absence of a nucleus (Hara et al., 1980; Sakai and Kubota, 1981; Newport and Kirschner, unpublished data). It has been shown that inhibiting protein synthesis in activated eggs results in blocking the egg at the end of S phase of the cell cycle (Harland and Laskey, 1980; Miale-Lye et al., 1983). When protein synthesis in dividing (pre-midblastula transition) eggs is inhibited (to at least 95% inhibition), the endogenous embryonic nuclei complete one round of DNA synthesis and begin swelling in size (Figure 6A; Miale-Lye et al., 1983). The result of this swelling is a nucleus whose volume is 5–20 times larger than the volume of a normal embryonic S-phase nucleus (Figure 6B). Focusing through such S-phase-blocked nuclei reveals that the DNA is not found in the interior but seems to be bound exclusively to the nuclear envelope (Figure 6A). When \( \lambda \) DNA is injected into a cycloheximide S-phase arrested egg, it assembles...
into nuclei through the same series of steps described earlier (Figure 6C). Once assembled, these nuclei behave identically to endogenous nuclei in that they swell in size relative to the λ-DNA-containing nuclei formed in un-blocked eggs (Figure 6C; compare with Figure 2B). The end result of this swelling is a nucleus with a large surface area, to which the λ DNA is attached. This result demonstrates, first, that all of the structural components and enzymatic activities necessary to assemble λ DNA into nuclei are present in excess in the egg, since no new translation is necessary for nuclear formation. We have found that there is enough of these “nuclear precursor” components stored in the egg to package at least 5–8 ng of λ DNA. This would be the amount of DNA contained in about 1000 Xenopus embryonic nuclei. Second, the nuclei formed with λ DNA respond in the same way to cycloheximide treatment as embryonic nuclei. Cycloheximide blocks embryonic nuclei at the end of S phase with a characteristic morphology (Mialke-Lye et al., 1983). Since λ-DNA-containing nuclei exhibit the same morphology, they are presumably responding to the same cell-cycle cues.

During mitosis, somatic and embryonic nuclei disassemble and the DNA undergoes condensation into metaphase chromosomes. Masui and coworkers have demonstrated that unfertilized, nonactivated Xenopus eggs contain a cytoplasmic component called cytoplasmic factor (CSF) which, when injected into the cells of dividing fertilized eggs, causes these cells to arrest in mitosis (Meyerhof and Masui, 1979a, 1979b). In order to determine whether nuclei containing λ DNA respond to the molecular signals that cause nuclear breakdown and chromosome condensation in normal nuclei, λ-DNA-containing nuclei were exposed to cytoplasmic factor. For this, λ DNA was injected into activated eggs and allowed to incubate for 2.5 hr, sufficient time for most of the λ DNA to be converted into nuclei with an appearance similar to the nuclei shown in Figure 2B. Following this incubation, the eggs were injected
Figure 5. Electron Microscopy of Nuclei Containing λ DNA

(A) Low magnification view of an intact λ-DNA-containing nucleus showing double nuclear membrane and surrounding cytoplasm (7000×). (B) Intermediate magnification of another λ-DNA-containing nucleus showing exclusion of cytoplasmic organelles from the nucleus and distribution of the nuclear pores (●) in the surrounding nuclear membrane (19,500×). (C) and (D) High magnification of the pore-membrane complex of a λ-DNA-containing nucleus (C) and an early embryonic nucleus from a 5 hr old embryo (D). Both (C) and (D) are magnified 72,000-fold.

with 100 nl of cytoplasm withdrawn from unfertilized non-activated eggs (CSF-containing cytoplasm). The CSF-injected eggs were incubated for 60 min prior to lysis. Control eggs were treated identically, but after injection of the DNA the eggs were injected with cytoplasm from activated eggs, which lacks CSF activity. When these control eggs were observed with phase and fluorescence microscopy the nuclei containing λ DNA were still intact. However, in eggs that had been injected with CSF we could no longer find such nuclei. Instead, highly condensed DNA lacking a nuclear envelope was found. Figure 7 shows the condensed λ DNA observed in CSF-injected eggs, as visual-
ized by fluorescence microscopy. No nuclear envelope was visible by phase contrast microscopy (not shown). The increased condensation of DNA caused by CSF injection can be seen by comparing Figure 7 with Figure 6C. Both the thickness of DNA fibers and the persistence length (the average distance between bends in the fiber) are much greater after exposure to CSF. Thus by arresting the egg in mitosis with CSF, the nuclear envelope once surrounding the λ DNA has been broken down, and the λ DNA has been structurally rearranged to a compact configuration loosely resembling condensed metaphase chromosomes. This "condensed" λ DNA was approximately 6 times thicker than DNA bound to the nuclear envelope in nuclei from non-CSF-arrested eggs (compare Figures 7 and 6C). That the nuclear lamins were no longer present around the λ DNA was confirmed by staining with antilamin antibody in an experiment identical to that described in Figure 4. In mitotically arrested eggs no staining was observed around the condensed λ DNA with anti-lamin antisera (data not shown). These experiments demonstrate that nuclei containing λ DNA respond to the molecular signals that induce endogenous nuclei to break down their nuclear envelopes and condense their chromosomes for mitosis.

Discussion

In this study we show that when DNA from a variety of procaryotic sources is injected into unfertilized Xenopus laevis eggs, it induces the formation of a vesicular structure around itself. We have shown via immunofluorescent staining that this envelope enclosing the DNA contains lamins, the major structural proteins present in the nuclear envelope of eucaryotic nuclei (Berezney and Coffey, 1974;
Dwyer and Blobel, 1976). Furthermore, the spontaneously formed envelope contains a double phospholipid bilayer and nuclear pores characteristic of normal nuclei. These results suggest that a large portion of the structural framework of the nucleus spontaneously assembles around the injected DNA. Assembly is either not dependent upon specific DNA sequence information or, if particular sequences are required, they would have to be quite small to occur at random in the 4.7 kb of pBR322 (less than 6 bp). Clearly, neither centromeric nor telomeric sequences are required. The data support a molecular mechanism whereby the reassembly of nuclear structure at the end of mitosis is initiated directly along the surface of the DNA molecules, presumably mediated by chromosomal proteins.

These results further show that the Xenopus egg contains a large pool of components necessary to assemble nuclei. This pool must be stored during oogenesis since blocking protein synthesis in the egg does not affect formation of nuclei. Stored components are present in quantities sufficient to package at least 6 ng of DNA into nuclear structures. This is equivalent to approximately 1000 Xenopus nuclei, assuming that the diploid DNA content of a Xenopus nucleus is 6 pg (Laskey et al., 1977). It has been shown that the egg stores enough histones during oogenesis to package about 20,000 nuclear equivalents of DNA into chromatin (Woodland and Adamson, 1977; Laskey et al., 1977). Small nuclear RNAs and snRNP proteins are also stored in large amounts in the egg cytoplasm (De Robertis et al., 1982; Zeller et al., 1983; Forbes et al., 1983). It has been suggested that the nuclear envelope and pore complexes may be stored in a pitted membrane complex in the egg called the annulate lamellae (Balinsky and Devis, 1963; Kessel, 1981). The storage of large pools of nuclear precursor components appears to be a developmental necessity in that the nuclear content of the egg rises from one nucleus at fertilization to 4000 nuclei 7 hr after fertilization (Gurdon and Woodland, 1969; Newport and Kirschner, 1982a). Using these stored pools rather than relying on de novo synthesis undoubtedly plays a major role in allowing the cell cycle to proceed at the accelerated rate that occurs during this period in development. We have recently proposed that this rapid cleavage period terminates after the 4000-cell stage due to the depletion of a component from the cytoplasm by the titration of this component onto DNA (Newport and Kirschner, 1982a, 1982b). Given the results presented here, the titratable component could well be a component indirectly bound to DNA via the nuclear envelope.

The presence of a large pool of nuclear precursor components within the egg demonstrates that such components are not sufficient for either initiation of nuclear assembly or nuclear growth. Our results suggest that initiation of nuclear envelope assembly occurs on or around the surface of chromatin, i.e., in the absence of DNA, the components do not form nuclei. Once initiated, however, it does not appear that nuclear envelope growth or nuclear volume is regulated exclusively by nuclear DNA content. Thus in eggs arrested at the end of S phase (by blocking protein synthesis), both early embryonic nuclei and nuclei containing λ DNA continue to swell in size. The DNA of these nuclei becomes very thinly spread out over the entire surface area of the nucleus. A possible explanation for this process is that the DNA template acts as a
catalytic surface, bringing several components together such that the nuclear envelope forms around the DNA. Once formed, however, the envelope containing the nuclear pore transport system could begin transporting “nuclear” proteins into the nucleus, causing it to swell. It has been shown that exogenously injected nuclear proteins such as histones and nucleoplasmin (proteins stored in large pools in the egg) are transported into the Xenopus laevis oocyte nucleus (Gurdon, 1970; Bonner, 1978; Dinglewall et al., 1982). The oocyte nucleus (germinal vesicle) is itself a good example of a very large nucleus (60 μl in volume) containing very little DNA (tetraploid, 12 pg). If true, this scheme would predict that initiation of nuclear envelope formation depends on the presence of a DNA template, but that growth of the envelope depends on selective transport of proteins or fluid into the nucleus. In the egg, the “transportable” proteins are present in excess quantities. Nuclei formed around injected DNA can therefore continue to increase in volume far beyond their normal size. In a normal embryo, nuclear size is presumably limited by the breakdown of the nucleus at mitosis, which occurs every 30 min. These observations are consistent with a number of other experiments demonstrating that nuclear size and structure change when nuclei are transplanted into different cytoplasmic environments (Gurdon, 1976; La Fond et al., 1982).

It should be noted that the results of these experiments suggest that any DNA injected into Xenopus eggs will be assembled into nuclear structures within 90 min after injection. Because this injected DNA is stable in the egg up through neurulation (24 hr after injection; Bendig, 1981; Rusconi and Schaffner, 1981; Newport and Kirschner, 1982b), it would be of interest to determine whether the induced nuclei fuse with the endogenous nuclei present at these latter times or whether they remain separate. The answer to this question would be important in evaluating many of the recent experiments demonstrating that exogenously injected cloned genes are transcriptionally expressed in a temporarily conserved manner during Xenopus development (Bendig, 1981; Rusconi and Schaffner, 1981; Newport and Kirschner, 1982b). Our results clearly demonstrate that such transcriptional processes can no longer be assumed to be taking place in the cytoplasm of the egg.

We have found that nuclei formed around λ DNA break down when the egg is arrested in mitosis by addition of cytotactic factor (CSF). This demonstrates that these spontaneously formed nuclei are not only structurally similar to endogenous nuclei but also contain the molecular components necessary for responding to the cues that regulate nuclear structure during the cell cycle. Furthermore, it appears that the λ DNA condenses into a thick filament resembling metaphase chromosomes when the egg is arrested in mitosis. This DNA lacks both centromeric and telomeric sequences. If this is true metaphase condensation, then it suggests that the process of chromosome condensation requires neither these elements nor specific DNA sequences. We also find that when λ DNA is injected into an egg arrested in S phase (via protein synthesis inhibition), nuclei form around the DNA. This finding indicates that nuclear formation does not require a mitotic (telophase) chromosome template. However, we cannot eliminate the possibility that chromosome condensation into a metaphase configuration is an intermediate along the pathway to nuclear formation under these conditions. That is, if chromosomes spontaneously condense into the metaphase configuration in the absence of the nuclear envelope, then λ DNA injected into an S-phase arrested egg might go through such an obligatory condensed stage before the nuclear envelope can begin forming around it.

The results presented here demonstrate that DNA injected into Xenopus laevis eggs is packed into nuclear structures that contain the major proteins of the nuclear envelope, nuclear pores, and a double nuclear membrane. It will be important in the future to determine whether these induced nuclear structures contain all of the components normally found in embryonic nuclei or whether some components are missing. The absence of a specific component might indicate that the component requires specific DNA sequence or secondary structure information in order to bind with high affinity to the nucleus. At present we feel that this system could be used to provide valuable information about the molecular mechanisms involved in both the regulated formation and breakdown of the nucleus in vivo and perhaps a way to study these processes in vitro.

**Experimental Procedures**

**Materials**

Bacteriophage λ DNA (λchip 57 Sam7) was prepared from phage particles purified in a CsCl gradient. The anti-laminate was a human anti-lamin obtained from a patient with linear spondylosis. The lamin has been shown to be specific for lamins A and C, both by immunofluorescence on tissue culture cells and by Western protein blots (McKeon et al., 1983), and to react with Xenopus lamin proteins (Macle-Lye et al., 1983). Mature Xenopus eggs were obtained from female frogs injected with 700 U human chorionic gonadotropin (Sigma) 8–12 hr before use, after priming the frogs for egg-laying 3–7 days previously with 100 U pregnant mare serum gonadotropin (Gestyl Organon, Ltd.; Newport and Kirschner, 1982a). Buffer A consisted of 15 mM Pipes, 2 mM EGTA, 2 mM EDTA, 80 mM KCl, 20 mM NaCl, 0.5 mM spermidine, 0.2 mM spermine, and 0.5 M sucrose (pH 7.2). MMR plus 5% Ficol is described in Newport and Kirschner (1982a). The fluorescent dye used to stain DNA was bisbenzimide (Hoechst-33258).

**Formation of Nuclear-Like Structures**

Bacteriophage λ DNA was injected into dejellied mature unfertilized Xenopus eggs (5–8 ng DNA in 75 nl of 10 mM potassium phosphate, 0.1 mM EDTA, pH 7). Injection causes activation of the eggs. The female haploid genome does not divide under these conditions. Injected eggs were placed on a glass slide at various times after injection and a small drop (10 μl) of 3.7% formaldehyde in Buffer A plus 10 μg/ml bisbenzimide Hoechst-33258 dye was added. After 2 min, the egg was lysed by addition of a coverslip. The state of the injected DNA, made fluorescent by binding of Hoechst dye to the major groove of the DNA, was examined with fluorescence microscopy, as well as with phase optics.

In some cases, it was necessary to reduce the concentration of yolk platelets surrounding the nuclei for better visualization of nuclear structure. In these cases, injected eggs were fixed in an Eppendorf test tube in 10 μl per egg of the above fixative by gently pipetting the egg through a large bore pipette tip one time, followed by a 5 min fixation. Repeated pipetting
of this solution through a pipette tip was performed to separate yolk platelets from the nuclei. The sample was allowed to sit for 10 min so that large aggregates of yolk could settle. Ten microliters of the supernatant was spotted on a slide, followed by addition of a coverslip and examination by fluorescence, and phase contrast microscopy.

**Immunofluorescence on Frozen Sections**

Unfertilized Xenopus eggs were injected with 5-8 ng of bacteriophage λ DNA in 75 nl of 10 mM potassium phosphate, 0.1 mM EDTA, (pH 7). After 5-6 hr, the injected activated eggs were transferred to 10 ml of 3.7% formaldehyde in phosphate-buffered saline (PBS) and fixed for 1 hr on ice. The fixed eggs were then washed overnight in PBS on ice. The eggs were frozen in OCT tissue-fixing media with CO₂ gas and sectioned in a cryostat. The 10 μm frozen sections, affixed to subbed glass slides, were incubated in primary anti-lamin antisera (diluted 1:100 in PBS, 1% BSA, 0.1% Triton-X100) for 15 min at room temperature. The slides were washed several times for 5 min in PBS-Triton buffer, before incubation in rabbit anti-human antisera tagged with rhodamine (diluted 1:100 in PBS plus 1% BSA). The sections were washed several times for 5 min in PBS-BSA and stained with bisbenzimide dye (10 μg/ml in PBS-BSA). The drained slides were then mounted under a coverslip in 90% glycerol, 0.15 M Tris- HCI (pH 7.4). Sections were photographed on Tri-X film. Anti-lamin staining of embryonic nuclei was performed with gastrula-stage embryos exactly as described above.

**Electron Microscopy**

Unfertilized eggs were injected with λ DNA (5-8 ng) and allowed to incubate for 2 hr. The eggs were fixed overnight at room temperature according to Kalland and Tandler (1971), before washing in buffer for 90 min. In some cases the animal pole was separated from the vegetal pole at this point. The eggs or egg fragments were placed in 1% osmium for 1 hr, washed, dehydrated, and embedded in Medcast embedding media (soft block mixture). After 24 hr of polymerization, 5 μ thick sections were cut through the egg with a glass knife. Sections were rescued and placed on a glass slide on a drop of 10% acetone, followed by heating on a slide heater. The sections were stained with a 1:1 mixture of 1% methylene blue in a borax buffer and 1% azur B in water and viewed under a Nikon phase scope at 40X to identify areas of injection of DNA. Selected sections were mounted by inverting a beam capsule containing araldite (hard block) over them. After 24 hr polymerization, thin sections were cut with a diamond knife and stained 15 min with 1% uranyl acetate and 5 min with lead citrate. The sections were viewed on a Phillips 400 electron microscope. Pre-membralula stage embryos were treated in the same manner for visualization of the pores of the embryonic nuclei.

**Autoradiography**

Bacteriophage λ DNA (c857) was end-labeled with [3H]-dATP using T4 DNA polymerase. Individual eggs were injected with 1 ng (600 cpm) of labeled DNA, incubated for 2 hr. Fixed in Telfestsincky's Smith media, dehydrated, embedded in paraffin, sectioned at 10 μm, dipped in photographic emulsion, and exposed for 1-3 days. Prior to examination the sections were stained with bisbenzimide dye.

**Acknowledgments**

We would like to thank Frank McKeon and Ryn Make-Lye for helpful discussions and communication of results prior to publication. We are especially grateful to Richard Hubbell for the electron microscopy. We would also like to thank Dr. Saty Zigmond for valuable discussions (and euphonious comments), Dr. Thomas Komberg for generous support, and Vivian Siegel for critical reading of the manuscript. This work was supported by a National Institute of Health postdoctoral fellowship (F32-GM07615-01) to J. N., an A. C. S. California Division Senior Postdoctoral Fellowship (#8-14-82) to D. F., and by grants to M. K. from the American Cancer Society and the National Institutes of Health.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received May 2, 1983

**References**


Spontaneous Formation of Nucleus-Like Structures


Note Added in Proof

Recent results show that reconstitution of an envelope-enclosed structure containing λ DNA also occurs in vitro (in an extract of Xenopus eggs).