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Chromosome replication in cell-free systems from *Xenopus* eggs

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[Plate 1]

Cell-free systems from eggs of the frog *Xenopus laevis* are able to perform most of the acts of eukaryotic chromosome replication *in vitro*. This now includes the crucial regulatory step of initiation, which had only been achieved for viral systems previously. Purified DNA or nuclei are able to initiate and complete semi-conservation replication in egg extracts *in vitro* (Blow & Laskey, *Cell* **47**, 557–587 (1986)). Replication does not require specialized DNA sequences either *in vitro* or in microinjected eggs, but in both systems large templates replicate more efficiently than small templates. In some cases replication can re-initiate, excluding the possibility that replication is primed by preexisting primers in the template preparations. When nuclei are replicated *in vitro*, only one round of replication is observed in a single incubation resembling the single round of replication observed for purified DNA after micro-injection. The mechanism that prevents re-initiation of replication within a single cell cycle is discussed and certain models are eliminated.

Nucleosome assembly from histones and DNA has also been studied in cell-free systems from *Xenopus* eggs. Fractionation has led to the identification of two acidic proteins called nucleoplasmin and N1, which bind histones and transfer them to DNA. The sequences of both proteins have been determined by cDNA cloning and sequencing. Both proteins are found as complexes with histones in eggs.

Introduction

The replication of eukaryotic chromosomes is more complicated than that of their prokaryotic counterparts in several respects. First, initiation occurs at many sites within the much longer chromosomes of eukaryotes. Secondly, initiations must be coordinated in such a way that each region of the chromosome is replicated once, but only once, within a single cell cycle. Re-initiation does not occur until the next cell cycle. Thirdly, the DNA of eukaryotic chromosomes is organized into nucleosomes, which are then organized into higher orders of folding. This introduces the two additional problems of replicating through such complex structures and of reassembling them on the two progeny duplexes after replication.

Progress in analysing eukaryotic replication has been restrained by the lack of efficient cell-free systems. In particular, cell-free systems have been unable to initiate non-viral replication efficiently, yet initiation is the crucial regulatory step.

Eggs of the frog *Xenopus laevis* offer a fruitful approach to this problem because they contain an extraordinary stockpile of materials involved in chromosome replication (Laskey *et al.* 1985). With *Xenopus* eggs it has been possible to develop cell-free systems that mimic most of the events at a replication fork (Richter *et al.* 1981; Mechali & Harland 1982), and recently it has been possible to achieve efficient initiation of replication *in vitro* (Blow & Laskey 1986).

The stockpile of materials required for replication is provided to sustain the remarkably rapid rates of cell division in early *Xenopus* embryos. In the time that a mammalian cell can divide once, the embryo of *Xenopus laevis* has formed more than 20000 cells. Two additional properties of *Xenopus* eggs make them excellent material for cell-free systems. First, they are obtainable in large amounts throughout the year. Second, they can be micro-injected easily so that the synthetic capabilities of the system can be determined by micro-injecting templates into intact cells. Micro-injection can also provide a valuable check on the biological significance of results obtained from the cell-free systems.

In this paper we start by considering the results from a novel cell-free system that initiates replication *in vitro* by using either nuclei or purified DNA as the template (Blow & Laskey 1986). We then consider studies of the sequence specificity of replication that have produced clear, but puzzling, evidence that replication in the *Xenopus* egg does not require specific DNA sequences. We review investigations of the mechanism that prevents re-initiation of replication within a single cell cycle and that show that it does not require *cis*-acting sequences, but that it can act independently of DNA sequence. We discuss possible models that could account for this. Finally, we consider another aspect of chromosome replication, namely the assembly of newly replicated DNA into the nucleosome subunits of chromatin. We summarize and discuss evidence that nucleosome assembly in *Xenopus* eggs is mediated by at least two acidic proteins that bind histones and transfer them to DNA.

INITIATION OF DNA REPLICATION IN A CELL-FREE SYSTEM FROM *XENOPUS* EGGS

Initiation of replication *in vitro* has been elusive. Although efficient initiation of viral replication has been achieved (Challberg & Kelly 1979; Li & Kelly 1984; Stillman & Gluzman 1985; Wobbe *et al.* 1985), until recently it has not been possible to observe efficient replication on non-viral, cellular templates *in vitro*. However, Lohka & Masui (1983, 1984) prepared extracts of activated *Xenopus* eggs by gentle centrifugation of intact eggs. When demembranated *Xenopus* sperm nuclei were incubated in these low-speed supernatants, their chromatin decondensed and became surrounded by a newly formed membrane. These reconstituted nuclei also incorporated tritiated thymidine into DNA. By using density substitution and pool measurements, we have subsequently shown that the incorporation observed in similar extracts represents semi-conservative replication of almost all of the template (figure 1) (Blow & Laskey 1986). DNA repair or partial strand synthesis was not detected and synthesis was inhibited by aphidicolin. Short pulse labelling experiments revealed 4 *S* fragments as expected for semi-discontinuous synthesis of Okazaki fragments. Before replication could be detected, the demembranated sperm nuclei decondensed and reformed nuclear membranes containing nuclear pores (figure 2, plate 1).

It is possible to visualize replication within nuclei incubated in this system by using biotinylated dUTP as a precursor and then probing with streptavidin conjugated to a fluorescent dye such as Texas red or fluorescein (figure 3, plate 1) (Blow & Watson 1987). In this way it is possible to study the kinetics of replication within a population of nuclei that share the same cytoplasmic environment. For example, figure 3 shows that some nuclei have incorporated biotinylated precursor extensively whereas others have not yet started to incorporate. A powerful extension of this approach is made possible by flow cytometry, which measures the intensity of fluorescence of individual nuclei. By comparing the fluorescence

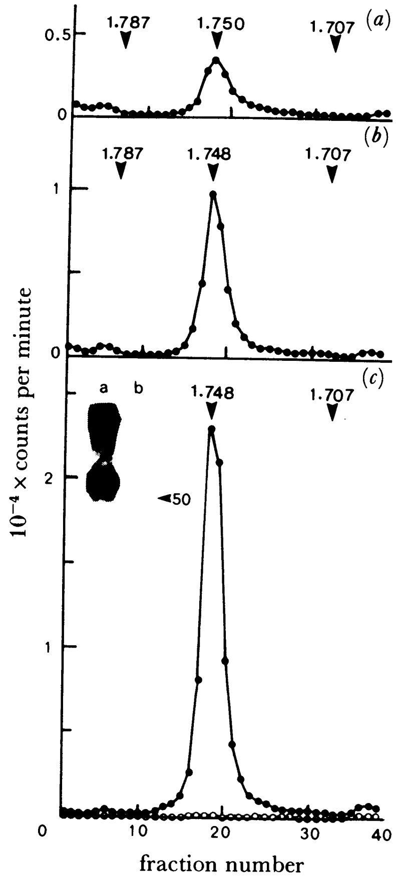


FIGURE 1. Density substitution of sperm nuclei incubated in the extract for 2, 4 and 6 h. Filled circles: sperm nuclei were incubated at a DNA concentration of 10 ng μl^{-1} in egg extract with [α-^{32}P]dATP and 0.25 mM BrdUTP for 2 (*a*), 4 (*b*), or 6 (*c*) h; the DNA was extracted and fractionated on CsCl density gradients. Figures above the arrows represent the CsCl density as measured by refractive index. Native *Xenopus* DNA bands at a density of approximately 1.71; *Xenopus* DNA substituted at half its thymidine residues by BrdUTP bands at a density of approximately 1.75. Open circles: 6 h incubation as above, but without addition of sperm nuclei (minus DNA control). Inset to (*c*): autoradiograph of agarose gel (4 g l^{-1}) of 6 h incubation. Lane a: conditions as for density gradient; lane b: as for density gradient, but supplemented with 10 μg ml^{-1} aphidicolin. Horizontal arrow shows position of 50 kilobase marker. (Reproduced from Blow & Laskey (1986).)

signals at two different wavelengths, it is possible to relate the extent of biotin incorporation to the increase in DNA content. Analysis of replication in this way has directly confirmed that nuclei double their DNA content during incubation in the egg extract and it has shown that individual nuclei behave as independent and integrated units of replication, even when they share the same cytoplasmic environment. A full account of this study is published elsewhere (Blow & Watson 1987).

REPLICATION OF PURIFIED DNA IN *XENOPUS* EGG EXTRACTS *IN VITRO*

When purified DNA is microinjected into intact *Xenopus* eggs, it replicates semi-conservatively (Gurdon *et al.* 1969; Laskey & Gurdon 1973; Ford & Woodland 1975; Harland & Laskey 1980). Also Forbes *et al.* (1983) found that injected DNA forms nucleus-like structures that are bounded by double membranes containing nuclear pore complexes and lined with lamins. Because DNA contained in the form of nuclei replicates (see above), the behaviour of purified DNA in the cell-free system was investigated to see if it would form nucleus-like structures and replicate. Figure 4, plate 1, shows that plasmid DNA incubated in the cell-free system becomes organized into discrete membrane-bound structures (Blow & Laskey 1986; Newmeyer *et al.* 1986). Electron microscopy reveals that these membranes contain nuclear pore complexes and closely resemble a normal nuclear envelope. Nuclear formation from plasmid DNA appears independent of DNA sequence; bacteriophage DNA or plasmid DNA (figure 4) is assembled into nuclei, but its efficiency is affected by the length of

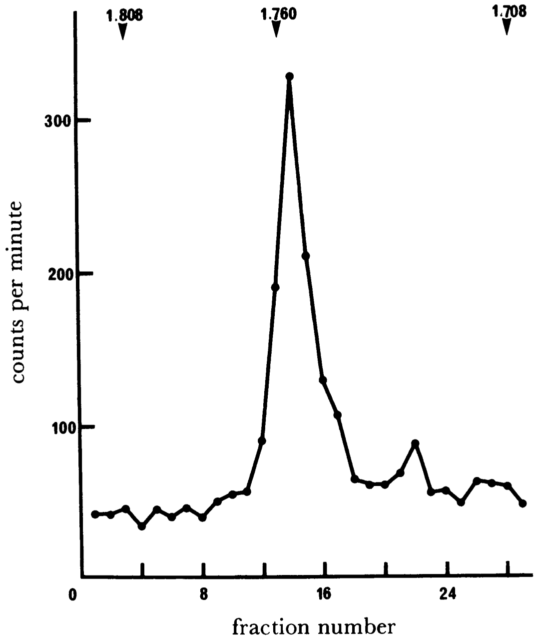


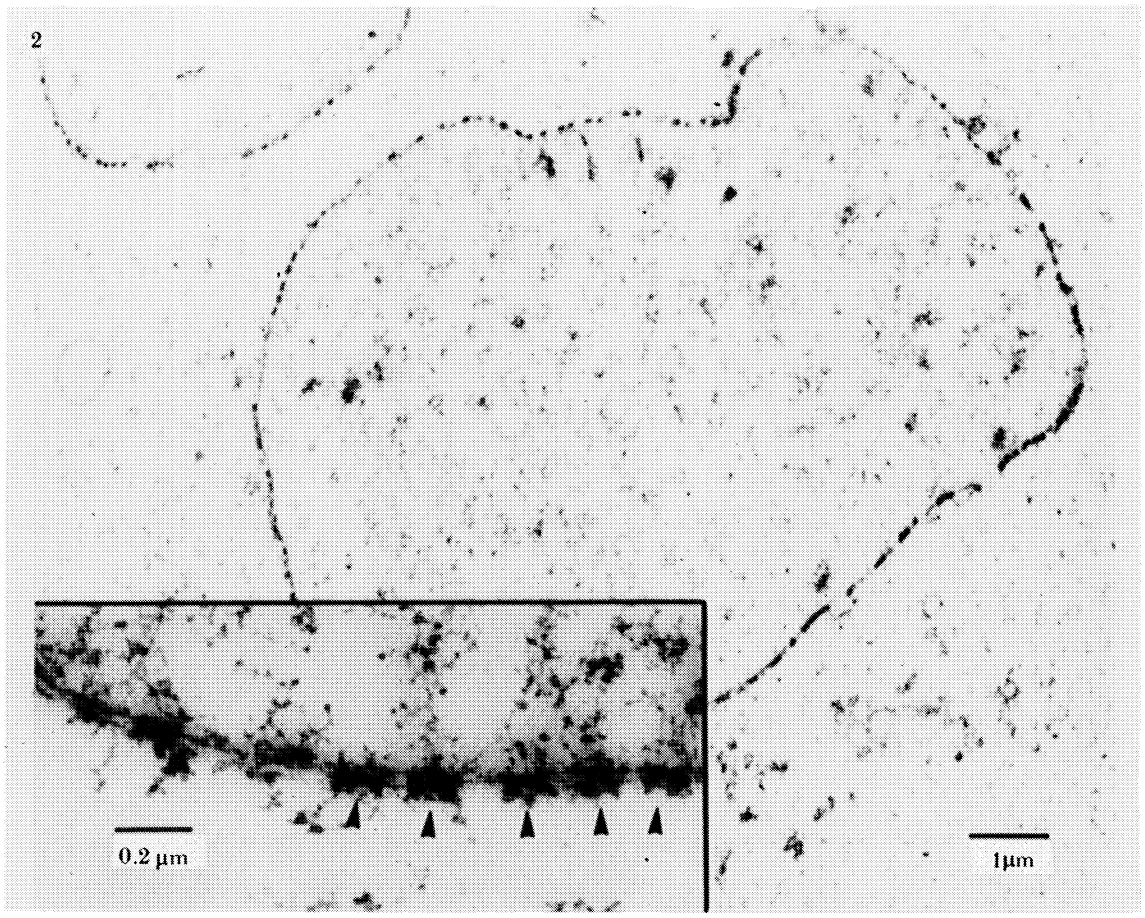
FIGURE 5. Density substitution showing replication of pBR322 *in vitro*. 200 ng pBR322 DNA was incubated in 20 μl of the *Xenopus* cell-free extract supplemented with [α-^{32}P]dATP and 0.5 mM BrdUTP for 6 h as previously described (Blow & Laskey 1986). DNA was extracted and fractionated on a CsCl density gradient. Arrows show gradient density as determined by refractive index.

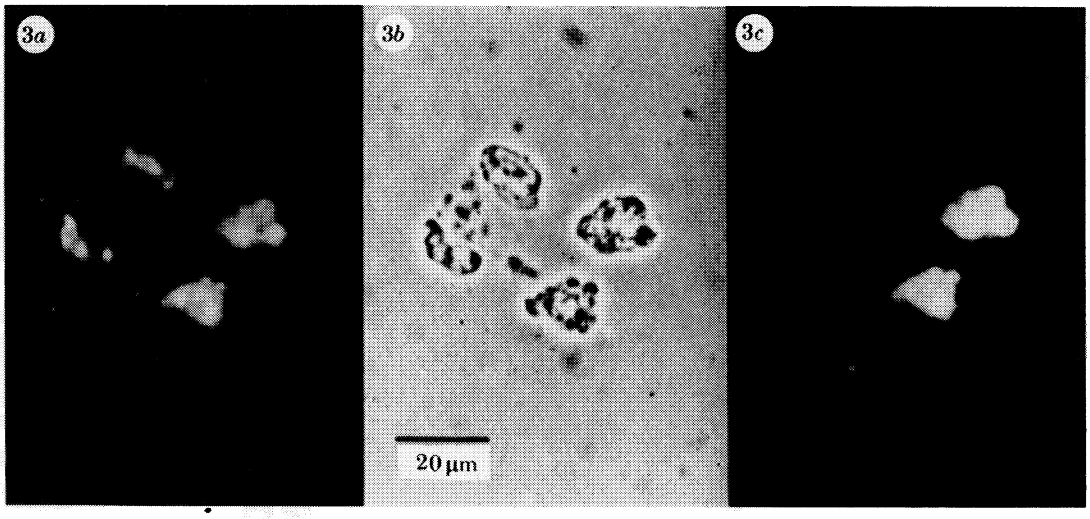
DESCRIPTION OF PLATE 1

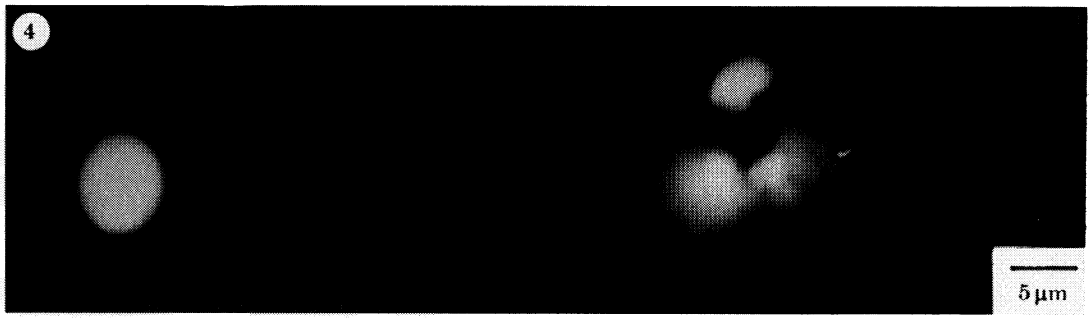
FIGURE 2. Electron micrograph of structure assembled *in vitro* from condensed, demembranated sperm nuclei. Highly condensed *Xenopus* sperm nuclei were demembranated and incubated in the *Xenopus* cell-free DNA replication extract for 8 h as described previously (Blow & Laskey 1986). They were then fixed in 50 volumes of tannic acid (2.5 g l^{-1}), paraformaldehyde (20 g l^{-1}), 0.1 M cacodylate buffer at pH 7.2, dimethylsulphoxide (20 g l^{-1}) and 1 mM $CaCl_2$. After pelleting (2100 *g*, Bench Centaur, 10 min), nuclei were washed, dehydrated and embedded in LR White (The London Resin Co., London, U.K.). Ultrathin sections were double stained with uranyl acetate and lead citrate (5 min each), and viewed by using a Phillips EM300. Inset: magnification of a portion of the envelope showing apparent nuclear pores (arrowed).

FIGURE 3. Incorporation of biotin into nuclei replicated *in vitro* in the presence of biotin-11-dUTP. Sperm nuclei were incubated with 40 μM biotin-11-dUTP in the cell-free replication extract for 4 h. They were then isolated as described previously (Blow & Watson 1987) and stained with Texas red-streptavidin (for biotin, and hence newly replicated DNA) and with Hoechst 33258 (for total DNA). Stained nuclei were viewed wet under fluorescence and phase-contrast optics. (*a*) Hoechst fluorescence; (*b*) phase contrast; (*c*) Texas red fluorescence.

FIGURE 4. Nucleus-like structures assembled *in vitro* from pBR322 DNA. 0.1 μg pBR322 DNA was incubated in 10 μl of extract for 4 h as previously described (Blow & Laskey 1986). DNA was stained with 10 μg ml^{-1} Hoechst 33258, and the sample was viewed wet under fluorescence optics.







Figures 2–4. For description see opposite.

the DNA. Short DNA molecules assemble into nuclei less efficiently than longer ones, though even pBR322 can form nuclei in the cell-free system (figure 4).

When purified DNA is added to the extract it is replicated, though less efficiently than is observed when nuclei are the template. Up to 38% of naked DNA templates have been observed to replicate, though the average falls between 5 and 10% (Blow & Laskey 1986). Density substitution experiments have shown that, as for nuclei, the incorporation seen with DNA templates represents semi-conservative synthesis of complete DNA strands and not partial strand repair. Figure 5 shows the result of a density substitution experiment with the prokaryotic plasmid pBR322 as the template. The possibility that synthesis occurs by priming from pre-formed primers in the DNA template, rather than by *de novo* initiation, is rendered unlikely by the observation that multiple rounds of replication can be observed under some circumstances (Blow & Laskey 1986).

INITIATION OF DNA REPLICATION IN *XENOPUS* EGGS DOES NOT REQUIRE SPECIFIC DNA SEQUENCES

When purified DNA is micro-injected into *Xenopus* eggs it replicates once per cell cycle (Harland & Laskey 1980). Surprisingly, it was found that replication does not require eukaryotic DNA sequences, but that a wide range of prokaryotic plasmids or viral genomes replicate. Templates that replicate include bacteriophages λ, M13, G4 and ϕX174, plasmids Col E1, pBR322, pMB9 and pAT153, together with a wide range of eukaryotic DNA fragments cloned into these vectors (Harland & Laskey 1980; McTiernan & Stambrook 1980; Mechali *et al.* 1983; Mechali & Kearsey 1984). In contrast, there have been claims of sequence-specific initiation of replication in *Xenopus* eggs (Watanabe & Taylor 1980; Chambers *et al.* 1982; Hines & Benbow 1982). A careful attempt to repeat these experiments (Mechali & Kearsey 1984) contradicted the claims of specific initiation and showed that replication is independent of DNA sequence, but depends on the size of the template DNA molecules. Large molecules replicate more efficiently and for templates between 4 and 11 kilobases there is a linear relation between template size and the amount of replication (Mechali & Kearsey 1984).

The sequence specificity of replication in the cell-free system has not been studied so systematically, but so far the results are consistent with those obtained from the intact egg. Large DNA molecules replicate more efficiently than small molecules *in vitro*, but apart from that no difference has been observed between templates of different DNA sequence. For example, figure 5 shows replication of the prokaryotic plasmid pBR322 in the extract *in vitro*. The most conspicuous difference in efficiency of replication between different types of template in the cell-free system is the difference between purified DNA and nuclei. Nuclei replicate about ten times more efficiently than purified DNA. This cannot be due to sequence differences between them because this difference is observed even when *Xenopus* sperm nuclei are compared with DNA purified from *Xenopus* sperm. A more likely explanation is that the DNA might have to be assembled into nucleus-like structures before it can replicate and that this step is inefficient. This interpretation is consistent with an observed correlation between nuclear reformation and replication for both purified DNA and demembranated nuclei (Blow & Laskey 1986). In both cases, replication has been observed only after nuclei have formed in the extract. Flow cytometry of biotin-labelled nuclei should help to reveal if this correlation is causal.

Experiments that investigate the replication of exogenous DNA templates cannot exclude the possibility that the endogenous chromosomes initiate at specific DNA sequences, but they raise questions about the possible functions of specific replication origins and allow two of the most obvious explanations to be excluded. First, they exclude the possibility that the enzymes of replication of the *Xenopus* egg are only able to initiate on specific eukaryotic replication origins, because all DNA molecules tested replicate (Mechali & Kearsey 1984). Second, they exclude the possibility that specific replication origins are required to coordinate multiple initiations within a chromosome in such a way that no region of the DNA can re-initiate replication within a single cell cycle. The evidence for this last statement and its implications for possible mechanisms for preventing re-initiation of replication within a single cell cycle are considered in greater detail in the following section.

MECHANISMS THAT PREVENT RE-INITIATION OF DNA REPLICATION WITHIN A SINGLE CELL CYCLE

With rare exceptions, eukaryotic chromosomes replicate each region of their DNA once and only once within a single cell cycle. Clearly, the cell must have a means of distinguishing replicated from unreplicated DNA to prevent re-initiation until the next cell cycle. A striking result from DNA injection experiments with *Xenopus* eggs is that replication of injected DNA is regulated by this mechanism so that it only replicates once in any cell cycle, but can replicate more than once when incubated through multiple cell cycles (Harland & Laskey 1980). Similarly, during a single incubation in the cell-free system, nuclei only replicate once without re-initiating; however, re-initiation is sometimes seen in a single incubation of purified DNA *in vitro* (Blow & Laskey 1986).

The mechanism that distinguishes replicated from unreplicated DNA to prevent re-initiation in a single cell cycle is not known, but certain classes of model can be excluded for the *Xenopus* egg. In the simplest type of model, re-initiation could be prevented by ensuring that all initiations occurred synchronously in a single burst at the start of S-phase. However, in *Xenopus* eggs *in vivo* and *in vitro*, and probably in other cells as well, initiation is asynchronous. Thus some molecules are seen to undergo complete density substitution whereas others have not entered the replicating intermediate pool (figure 1) (Harland & Laskey 1980; Blow & Laskey 1986). A similar conclusion is reported elsewhere for whole nuclei (Blow & Watson 1987).

An alternative type of model involves marking replicated DNA with an inhibitory signal which instructs the replication apparatus that a site has been replicated. The simplest variant of this class of model would involve marking replication origins that have been used so that they would not be used again until the next cell cycle (figure 6*a*). However, this would not provide a satisfactory explanation of the results from *Xenopus* egg injection experiments, because the mechanism that prevents re-initiation does not require specific DNA sequences in the *Xenopus* egg. All molecules that have been tested so far have replicated only once within a single cell cycle. This has been observed for a range of prokaryotic templates including bacteriophages G4, M13 and λ (Harland & Laskey 1980; Laskey *et al.* 1981; Mechali *et al.* 1983), even though their replication is not normally subject to this regulatory mechanism. This means that any negative regulatory mechanism that marks DNA with an inhibitory signal to prevent re-initiation must be pervasive and must mark the entire length of replicated DNA (figure 6*b*). It would not be sufficient to mark just the origin of replication, because replication could

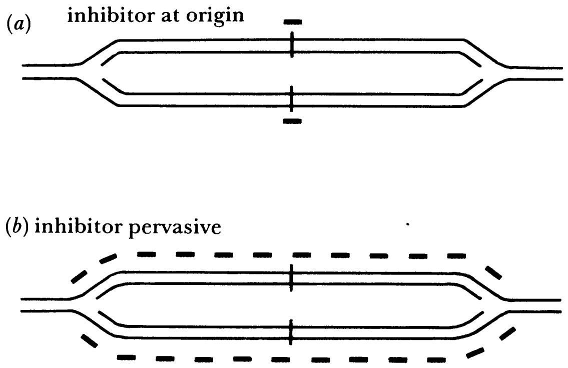


FIGURE 6. Negative inhibitor models for preventing re-initiation of replication in a single cell cycle. (*a*) After initiation the origin of replication becomes marked by an inhibitory molecule that prevents re-initiation at that site. (*b*) The entire region of replicated DNA becomes marked by a hypothetical inhibitor, rather than just the origin. Note that model (*a*) is inadequate to account for the results from *Xenopus* eggs (see text).

initiate at many alternative sites on the DNA molecule. For example, Harland & Laskey (1980) found that all six of the *Hin*dIII fragments of SV40 DNA can initiate replication after injection into *Xenopus* eggs, a conclusion supported by the electron microscopic observations of McTiernan & Stambrook (1984). Nevertheless, SV40 DNA replicates only once in a single cell cycle in the egg (Harland & Laskey 1980).

Alternatively, re-initiation could be prevented by a requirement for a positive regulatory activator before the start of the S-phase, as illustrated in figure 7 (Laskey *et al.* 1981). This model envisages synthesis of an unstable positive activator that is required for subsequent initiation of DNA replication, a 'licence to replicate'. The activator would be stabilized by binding to DNA, but any that remains unbound would be degraded before DNA synthesis starts. It would be inactivated either by use to initiate replication or by passage of the replication fork past the site to which the activator is bound. In this way the activator could persist on unreplicated DNA to allow delayed initiations, but no free activator would be available to allow re-initiation until after mitosis when the next period of activator synthesis would occur (figure 7).

Although existing experiments do not allow us to distinguish between positive and negative models for the mechanism that prevents re-initiation, they do allow us to refine or exclude certain possibilities. For example, we have seen above that negative models that mark only the origin of replication are inadequate to account for the ability of this mechanism to operate without specific eukaryotic DNA sequences in the *Xenopus* egg. Instead, it is necessary to invoke a pervasive signal that marks all the replicated DNA. We can also exclude the possibility that such a signal is provided by the pattern of CpG methylation of the DNA at *Hpa*II sites. Thus Harland (1982) found that unmethylated DNA or DNA that had been methylated at *Hpa*II sites (the internal C at CCGG) replicated equally and that the pattern of methylation was copied so that the progeny duplexes of unmethylated DNA remained unmethylated but progeny duplexes of methylated DNA had the same methylation patterns as the parental duplexes. Arrest of the cell-cycle clock by cycloheximide failed to disrupt this inheritance pattern even though it completely abolished re-initiation. In this case, replicated and unreplicated DNA still had indistinguishable patterns of methylation even though the egg was able to distinguish them to prevent re-initiation on replicated templates while allowing

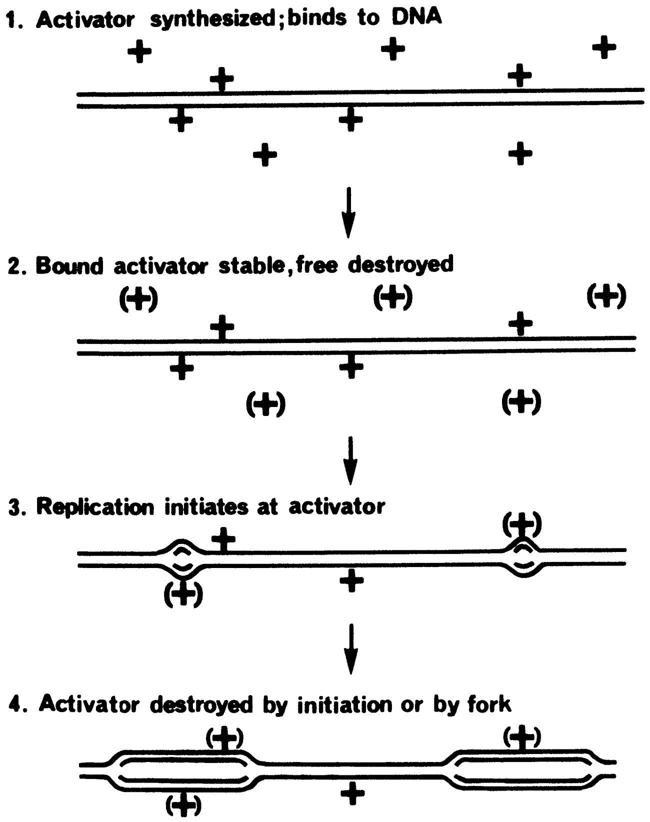


FIGURE 7. A positive activator model for preventing re-initiation of replication in a single cell cycle (redrawn after Laskey *et al.* (1981)). An unstable activator is synthesized at a single time before the start of S-phase. It is stabilized by binding to DNA, but any activator that remains unbound is degraded before the S-phase starts. Replication initiates only at the site of the activator, and either initiation or passage of the replication fork would destroy the activator In this way no activator would be available for re-initiation until more is synthesized in the next cell cycle. This model has the advantage that it is fail-safe as its failure would not result in illegitimate re-initiation.

unreplicated DNA to initiate at the same time. The data of Harland (1982) also make it unlikely that methylation at other CpG sites could provide a mechanism to prevent re-initiation. Because other DNA modifications are rare in eukryotes it seems more likely that a pervasive negative inhibitor of re-initiation would involve chromosomal proteins or perhaps modifications of proteins.

Recent studies of the replication of plasmids containing two different viral replication origins have led to the suggestion that the prevention of re-initiation within a cell cycle requires *cis*-acting DNA sequences (Berg *et al.* 1986; Roberts & Weintraub 1986). In the presence of the virus-coded T antigen the replication origin of SV40 is normally able to replicate many times within a single cell cycle, but when it is ligated to the replication origin of bovine papilloma virus it only replicates once per cell cycle. This has been interpreted as indicating that the papilloma virus origin is re-imposing the normal cellular control mechanism, but the possibility remains that one specialized viral mechanism is simply being replaced by another. We question the generality of these findings because the *Xenopus* egg does not require specialized eukaryotic DNA sequences to regulate DNA replication to only once per cell cycle.

Although it is not yet possible to determine whether re-initiation is prevented by positive or negative regulatory mechanisms, an advantage of a positive activator ('licence') model (figure 7) over negative regulatory models (figure 6) is that it would be fail-safe. Failure of a positive licence would result in only a gap in the pattern of initiations and this could be filled by convergence of the adjacent replication forks. In contrast, failure of a negative inhibitory model would result in illegitimate re-initiation, possibly causing gene amplification or even chromosome breakage if the additional replication forks progress past the centromere. The development of a cell-free system that displays this behaviour (Blow & Laskey 1986) should facilitate studies of the crucial mechanism that prevents re-initiation within a single cell cycle.

NUCLEOSOME ASSEMBLY IN CELL-FREE SYSTEMS FROM *XENOPUS* EGGS

A second aspect of eukaryotic chromosome replication that can be analysed by using eggs of *Xenopus laevis* is the assembly of DNA into the nucleosome subunits of chromatin. When purified DNA is added to high-speed supernatant fractions of *Xenopus* eggs, it becomes assembled into regularly spaced chains of nucleosomes (Laskey *et al.* 1977). The capacity for nucleosome assembly is remarkable. The homogenate from a single, mononucleate egg can assemble a mass of DNA equivalent to between 6000 and 12000 diploid nuclei into nucleosomes in only 1 h in the absence of protein synthesis. The histones required for assembly on to DNA are preformed and stored during oocyte growth before the phase of rapid DNA replication that follows fertilization (Adamson & Woodland 1974; Woodland & Adamson 1977). However, histones are not the only components involved in nucleosome assembly that are stored in *Xenopus* eggs. Although nucleosome cores can self-assemble from histones and DNA under some circumstances (reviewed by Laskey & Earnshaw (1980)), the *Xenopus* egg also contains at least two acidic proteins that act as assembly factors by binding histones and transferring them to DNA. These allow nucleosome cores to assemble rapidly at physiological ionic strength without forming insoluble aggregates.

The existence of such factors was postulated to explain why the stored histone pool of *Xenopus* eggs behaves as if the histones are in negatively charged complexes, even though histones are among the most positively charged proteins in the cell. Also, the fractions containing the negatively charged complexes were highly efficient in nucleosome core assembly (Laskey *et al.* 1978*a*, *b*). Fractionation led to the purification of an acidic, thermostable protein, 'nucleoplasmin', which is able to bind histones *in vitro* and to transfer them to DNA to form nucleosome cores. However, nucleosome core assembly by nucleoplasmin fails to mimic the unfractionated system in two ways. First, it fails to assemble the full complement of nucleosomes on to DNA and second, it requires very sharp optimum ratios of histones, nucleoplasmin and DNA. These shortcomings suggested that additional factors might be involved and Kleinschmidt *et al.* (1984) have now shown that additional acidic proteins are involved in binding histones H3 and H4. These proteins are called N1 and N2; they were discovered by Bonner (1975) by their ability to enter the nucleus rapidly after injection into the cytoplasm, a property they share with nucleoplasmin.

The properties of nucleoplasmin and N1 are summarized in table 1. The relationship of N1 to N2 is not clear. Although they electrophorese as two spots on two-dimensional gels, a tendency of N1 to degrade to a fragment resembling N2 makes it difficult to be certain that N1 and N2 are two separate polypeptides that both bind histones. The sequences of nucleoplasmin and N1 have both been determined recently from copyDNA (cDNA) clones (Dingwall *et al.* 1987; Kleinschmidt *et al.* 1986). They are both very rich in charged amino acids and both show extensive patches of acidic residues that could form histone binding sites. These are depicted in figure 8.

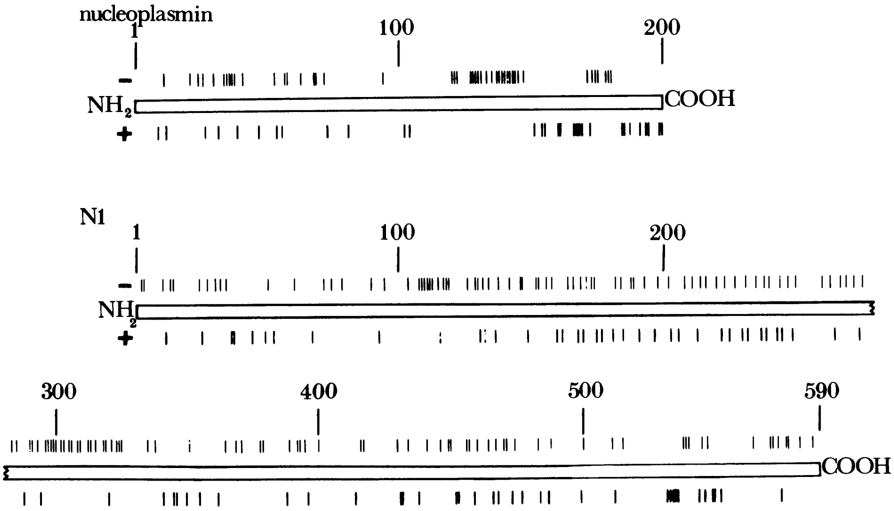


FIGURE 8. Distribution of charged amino acids in the nucleosome assembly factors nucleoplasmin and N1. Amino acid sequences were determined from cDNA clones and are depicted by rows of vertical lines. The centre box depicts neutral amino acids, the top row (–) depicts the acidic amino acids aspartate and glutamate, and the bottom row (+) depicts the most basic amino acids lysine and arginine. The sequence of N1 is displayed in two halves because of its much greater length. (Compiled from data of Dingwall *et al.* (1987); Kleinschmidt *et al.* (1986).)

TABLE 1. PROPERTIES OF THE NUCLEOSOME ASSEMBLY PROTEINS, NUCLEOPLASMIN AND N1

nucleoplasmin	N1
5 × 22000 KDa	67000 KDa
'30000' on gels	'110000' on gels
I.E.P. 4.5–5.5	I.E.P. 4.5
phosphorylated	
binds H2a and H2b	binds H3 and H4

Kleinschmidt *et al.* (1984) were able to demonstrate the coexistence of two classes of histone storage complex in *Xenopus* oocytes: (1) histones H3 and H4 bound to N1 and N2 and (2) histones H2a and H2b bound to nucleoplasmin, with two other histone-like bands that are also bound to nucleoplasmin. The identity of these other two proteins remains to be determined. They co-electrophorese with H3 and H4 on SDS–polyacrylamide gels, but other features such as iso-electric focusing behaviour suggest that they may not be H3 and H4. The identity of these two proteins is important if we are to be able to understand the steps in the nucleosome assembly pathway.

CONCLUSIONS AND PROSPECTS

With the ability to initiate replication in *Xenopus* egg extracts it has become possible to mimic, and therefore to analyse, most of the features of chromosome replication *in vitro*. Thus extracts are now able to initiate, elongate and terminate replication of purified DNA. They are also able to assemble DNA into regularly spaced chains of nucleosomes and to organize them into discrete nuclear structures surrounded by functioning nuclear envelopes. The value of such systems lies in the opportunity they provide for analysing the mechanism and regulation of chromosome replication. It is unlikely that all of the conclusions that emerge from studies of *Xenopus* eggs will be applicable to other types of cell, because the egg has adaptations that allow it to replicate its chromosomes exceptionally fast. These include the large stored histone pool and large stockpiles of other replication components. It is also likely that the remarkable lack of DNA sequence specificity for initiation of replication in the egg is an adaptation to the rapid rate of replication. Embryos of amphibians and insects initiate replication at closer intervals on their DNA than adult cells of the same species (Callan 1972; Blumenthal *et al.* 1974), allowing cell cycles of 35 min in *Xenopus laevis* or only 11 min in *Drosophila melanogaster*. It would be interesting to know if injected DNA would require specific sequences to initiate in cells of later embryos or adults that replicate more slowly and which initiate replication at longer distances on their DNA. Similarly, it would be interesting to know if the nucleosome assembly factors of *Xenopus* eggs are used in other types of cell.

For the present, uncertainties of this sort prevent us from generalizing from the *Xenopus* egg to other types of cell. Nevertheless, the cell-free systems derived from eggs of *Xenopus laevis* provide an exceptional opportunity to analyse both the mechanism and control of chromosome replication in a eukaryotic cell.

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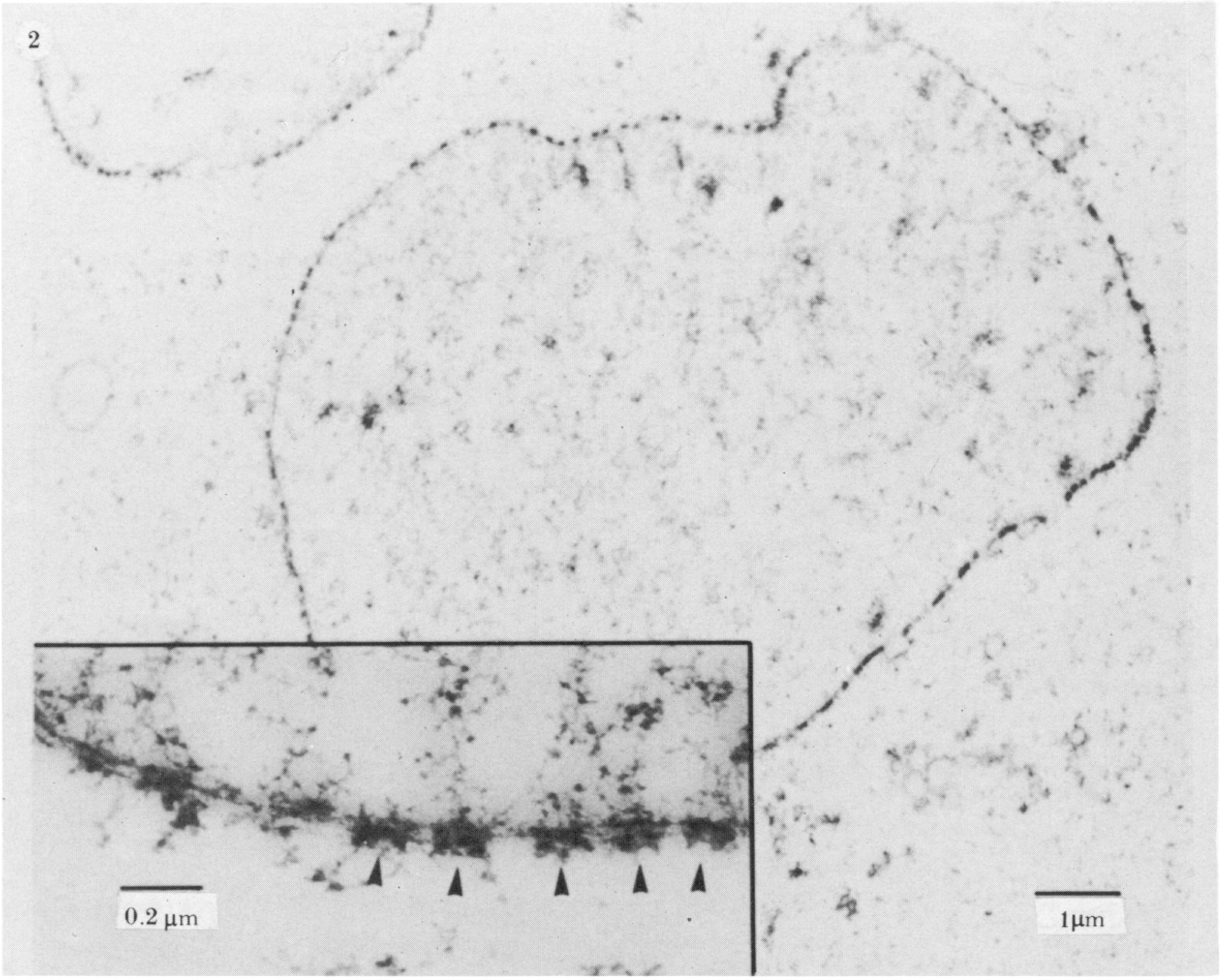

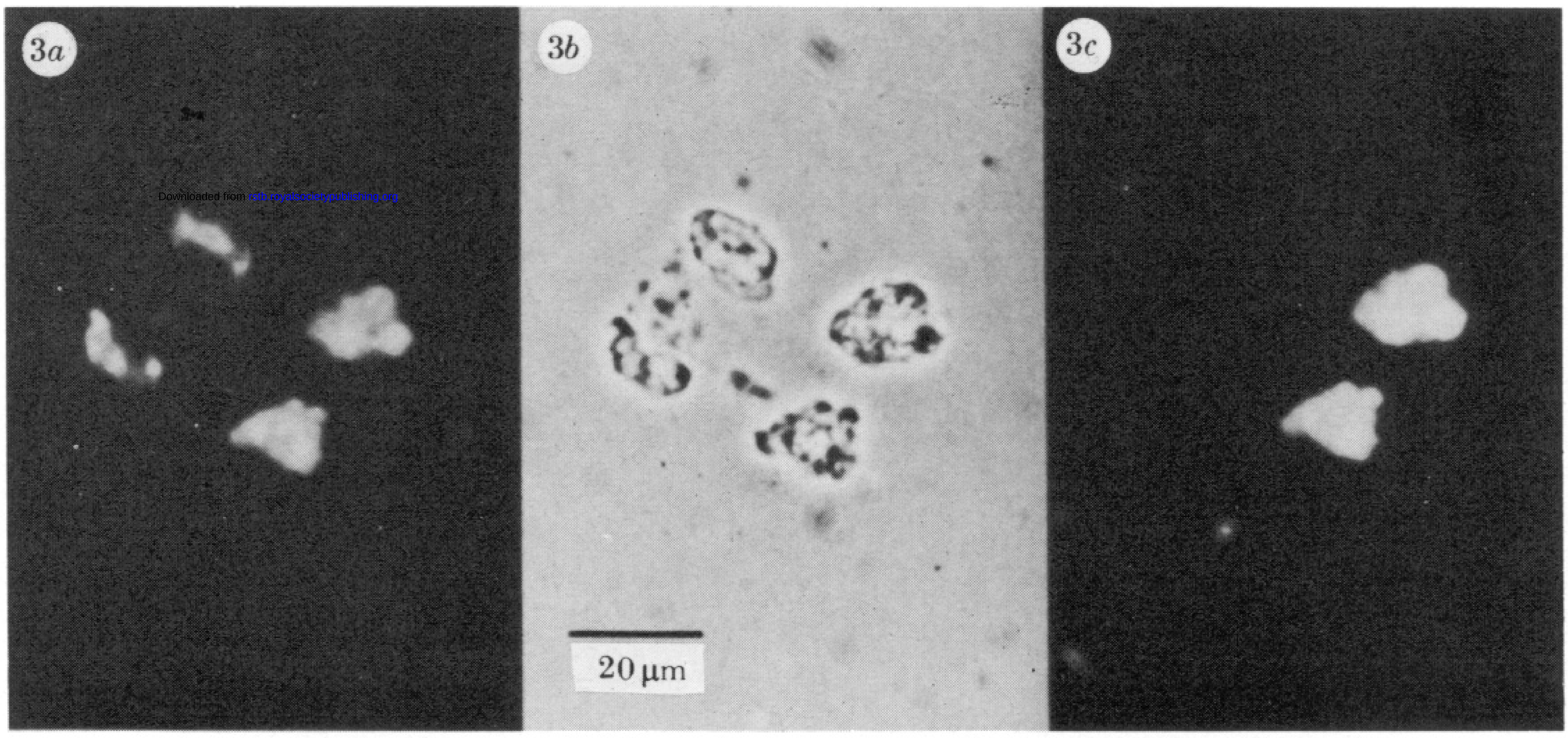

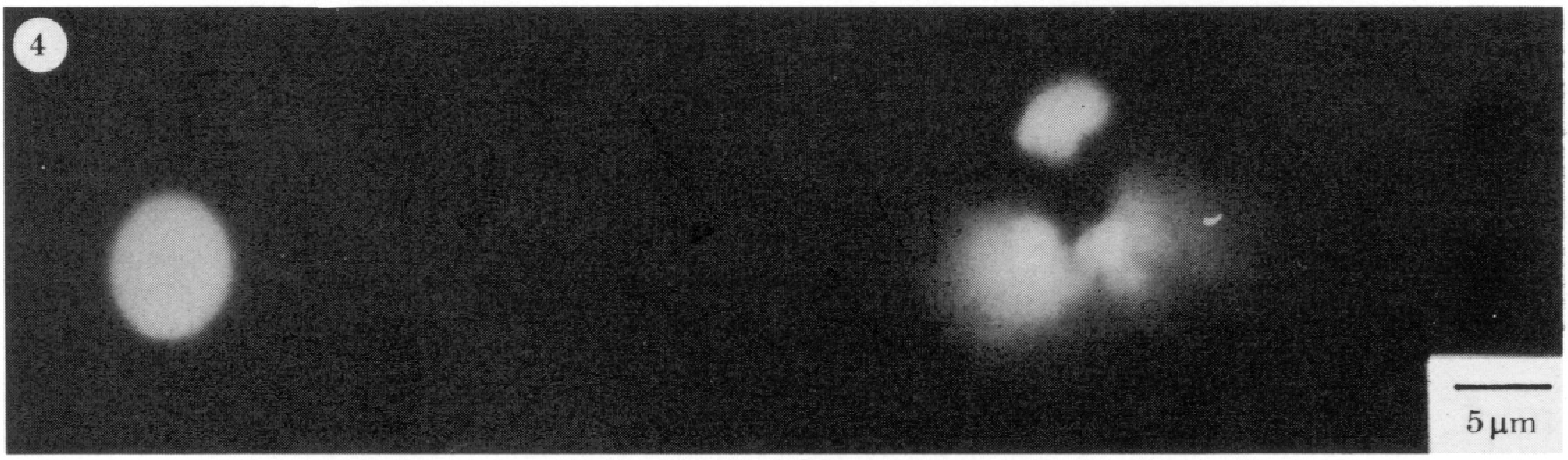

FIGURES 2–4. For description see opposite.