Efficient Plasmid DNA Replication in Xenopus Egg Extracts Does Not Depend on Prior Chromatin Assembly*

(Received for publication, August 21, 1995, and in revised form, September 25, 1995)

J. Aquiles Sanchez‡, Diane R. Wonsey, Leia Harris, Joanella Morales, and Lawrence J. Wangh
From the Department of Biology, Brandeis University, Waltham, Massachusetts 02254

Small plasmids replicate efficiently in unfertilized Xenopus eggs provided they are injected before rather than after activation of the cell cycle. Here we use Xenopus egg extracts to test the hypothesis that efficient replication results from chromatin assembly prior to activation giving preloaded plasmids a head start toward the formation of a replicating pseudonucleus (Sanchez, J. A., Marek, D., and Wangh, L. J. (1992) J. Cell Sci. 103, 907–918). As in ovum, plasmid DNA preincubated in unactivated egg cytoplasm (cytostatic factor extracts) replicate more efficiently after extract activation than does the same DNA added to the same extract after activation. Unlike in ovum, however, plasmids that replicate efficiently in vitro do not assemble into chromatin during preincubation and become topologically knotted instead. But even DNA knotting does not explain subsequent efficient replication. Also, plasmids preassembled into chromatin in vitro do not replicate efficiently in activated egg cytoplasm unless first preincubated in a CSF extract. We conclude that unactivated eggs contain replication-enhancing activities that can act independently of plasmid chromatin assembly and DNA topology. These postulated “preloading” factor(s) may be related to licensing factor, an activity that controls initiation of DNA replication in eukaryotic cells. The experimental conditions described here will permit characterization of preloading/licensing factor(s) in the context of a small plasmid substrate.

Chromatin assembly plays a central role in the pathway of nuclear formation leading to plasmid DNA replication in Xenopus eggs and egg extracts (1). Newport (2) first showed that plasmids assembled into chromatin bind nuclear membrane vesicles that fuse to form a nuclear envelope, complete with pores and lamina. The resulting pseudonuclei are essential for initiation of plasmid DNA replication (3, 4).

Our previous studies also pointed to early chromatin assembly as an important step leading to plasmid replication in intact eggs. We have shown that plasmids replicate efficiently in activated eggs, provided they are injected before rather than after the start of the first cell cycle (5). We have called this phenomenon the preloading effect and have correlated both the amount and the timing of plasmid replication to the extent of chromatin assembly prior to activation (6). We have suggested that chromatin assembly before the start of the cell cycle leads to efficient replication after activation because it gives molecules a head start toward the formation of pseudonuclei.

But there may be other reasons why incubation of a plasmid in the cytoplasm of an egg arrested in meiotic metaphase II leads to efficient replication. For instance, these eggs may contain enzymatic activities or DNA binding proteins other than histones that can directly enhance replication initiation after activation. This possibility is in accord with our observation that plasmid molecules injected into eggs that have already entered the first cell cycle show increased replication during the second cell cycle (i.e. after passage through the first mitosis) (6). It also fits the “licensing factor hypothesis” put forward by Blow and Laskey (7). These investigators argue that eukaryotic nuclei normally replicate only once per cell cycle because they have to pass through mitosis, or at least experience nuclear envelope breakdown, before they can initiate DNA synthesis a second time.

Given these alternative possibilities, we decided to directly determine whether prior chromatin assembly actually accounts for efficient plasmid replication after the start of the cell cycle. In order to facilitate this analysis we first identified conditions for efficient plasmid DNA replication in vitro. We report here that efficient in vitro replication, like efficient in ovum replication, depends on exposure of plasmid to cytoplasm of unactivated Xenopus eggs (CSF2 extracts). But contrary to our original hypothesis, chromatin assembly in this cytoplasm is not required for subsequent efficient plasmid replication. This observation was confirmed by attempting to replicate preassembled plasmid chromatin directly in activated egg extracts. Once again we observed that exposure of the template to the unactivated egg cytoplasm is required for subsequent efficient replication, regardless of the initial degree of plasmid chromatin assembly.

In the course of this investigation, we also discovered that CSF extracts cause closed circular plasmid molecules to become topologically knotted. This observation led us to examine whether DNA knotting in unactivated egg cytoplasm might account for efficient in vitro replication. Our results establish that neither chromatin assembly nor DNA knotting before the start of the cell cycle accounts for subsequent efficient plasmid replication. We conclude that the preloading effect is most likely due to additional specific DNA-protein interactions in unactivated egg cytoplasm. A possible candidate for these preloading factor(s) is replication licensing factor, an activity responsible for cell cycle regulation of DNA replication whose components have started to be identified recently (8–10). The in vitro system described here will permit characterization of this and other replication-enhancing activities of the mitotic egg cytoplasm in the context of an easily characterized plasmid substrate instead of the complex genome of whole eukaryotic nuclei.

*This work was supported by Grant 3206 from the Council for Tobacco Research (to L. J. W.). 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.
‡To whom correspondence should be addressed. Tel.: 617-736-3111; Fax: 617-736-3107; E-mail: Sanchez@binah.cc.brandeis.edu.

1 A. Aguilar and L. J. Wangh, unpublished observations.
2 The abbreviation used is: CSF, cytostatic factor.
Role of Prior Chromatin Assembly in Replication Efficiency

MATERIALS AND METHODS

In Vivo Analysis of Plasmid DNA Replication—In vivo analysis of FV1 DNA replication and chromatin was carried out as described previously (6).

Extraction Preparation—Low speed extracts from metaphase arrested eggs (CSF extracts) were prepared according to the protocol of Wang et al. (11) and were used fresh rather than frozen and thawed. Exacts were supplemented with a final concentration of 10 mM creatine phosphate, 10 μM creatine kinase, and 0.1 mM CaCl_2. When specified, 50 μM PO_4 was added to a final concentration of 80 μM. At the times indicated in the text, the CSF extract was induced to enter the cell cycle by the addition of calcium to a final concentration of 1.2 mM CaCl_2, as measured by a precipitous decline in H1 kinase activity. Activation with 3 mM CaCl_2 as recommended by Blow and Sleeman (3) was found to impede progress of the cell cycle in the extract (data not shown).

To prepare high speed extracts from activated eggs, eggs were activated for 28 min at 20°C and then processed as described bywang et al. (11). The resulting low speed supernatant was further centrifuged in a SW50.1 rotor for 30 min at 45,000 rpm. The cytoplasmic layer was spun once more at 45,000 rpm for 30 min, adjusted to 7.5% glycerol (v/v), and then frozen in 20 μl aliquots in liquid nitrogen.

DNA Isolation from Egg Extracts—In a typical experiment, 2–10 μl aliquots of extract containing FV1 plasmid DNA were rapidly frozen on dry ice and were subsequently thawed and thoroughly dissolved by the addition of an equal volume of GuHCl buffer (4.5 mM guanidine-HCl, 0.1 mM EDTA, pH 8.0, 0.15 mM NaCl, and 0.05% sarkosyl) (5, 6). The salt concentration was then decreased by addition of 150 μl of STE buffer (150 mM NaCl, 50 mM Tris-Cl, pH 8.0, and 50 mM EDTA, pH 8.0) supplemented with 20 μg/ml of RNAse as a carrier, followed by the addition of 200 μl of protease K buffer (1% STE, 1% sarkosyl, and 1 ng/ml protease K). After incubation at 37–50°C for 1–2 h, the samples were extracted twice with phenol-chloroform, followed by ethanol precipitation and resuspension in TEEP buffer (10 mM Tris-Cl, pH 8.0, 1 mM EDTA, pH 8.0, and 100 μg/ml RNase A).

For isolation of non-nicked knotted plasmid molecules, the above protocol was modified to allow for closure of topoisomerase II-dependent nicks and double strand breaks (12). Aliquots of extract were adjusted to 0.5 mM NaCl and were incubated at room temperature for 30 min prior to freezing on dry ice. Subsequent DNA isolation steps were carried out as described above.

Analysis of DNA Replication and Chromatin Assembly—FV1 replication and chromatin assembly were measured by the DpnI resistance assay and the supercoiling assay as described previously (6). Southern hybridization signals were quantitated using a PhosphorImager (Molecular Dynamics), and replication efficiencies were calculated as the ratio of DpnI-resistant (replicated) material to the total amount of DNA/sample.

Generation, Assessment, and Replication of Chromatin Templates—FV1 DNA was added to a frozen and thawed high speed activated extract at a concentration of 16 ng/ml, and multiple aliquots were collected at regular intervals and frozen immediately on dry ice. The DNA from one aliquot at each time was purified and used to assess the level of chromatin assembly by examining the appearance of negatively supercoiled DNA in agarose gels containing 20 μg/ml chloroquine (6). In order to determine how efficiently each chromatin sample replicated, another aliquot was thawed and diluted 1:8 into a freshly prepared CSF extract either 10 min before or 10 min after activation of the extract by addition of calcium.

Characterization of Nicked Knotted and Knotted DNA Molecules—Nicked knotted DNA molecules migrate very differently from negatively supercoiled molecules on one- and two-dimensional chloroquine gels (13–15). One-dimensional chloroquine gels were carried out as described before (6). For two-dimensional chloroquine gel electrophoresis (16), 1 ng of knotted DNA was mixed with 1 ng of a collection of all possible FV1 negatively supercoiled topoisomers generated according to Bowater et al. (17). This DNA mixture was fractionated in the first dimension in a 0.72% agarose gel in 1× TPE (50 mM Tris, 10 mM EDTA, pH 7.2, with 85% phosphoric acid) buffer containing 0.75 μg/ml chloroquine at 70 V for 18 h. The gel was then equilibrated in 1× TPE buffer supplemented with 4.5 μg/ml chloroquine for 7 h and was run in the orthogonal dimension at 70 V for 16 h. Buffer was recirculated in both the first and second dimensions. Fractionated molecules were visualized by Southern transfer, hybridization, and autoradiography as described before (6).

RESULTS

The Preloading Effect in Ovum—Injection of plasmid DNA into unactivated eggs prior to the start of the cell cycle dramatically increases the efficiency of replication after cell cycle activation. FV1 molecules injected into unactivated Xenopus eggs do not replicate and therefore remain DpnI-sensitive (Fig. 1, lane A). But a high percentage of these molecules replicates during the first S phase after egg activation. As a result, full-length DpnI-resistant molecules accumulate and DpnI-sensitive molecules decrease (Fig. 1, lane B). In contrast, very few FV1 molecules injected directly into activated eggs replicate during the first S phase (Fig. 1, lane C), although a significant percentage of these molecules do replicate in the second S phase (data not shown, but see Ref. 6).

Why do plasmids preloaded in unactivated eggs replicate efficiently after activation? Once in the unactivated egg, negatively supercoiled molecules first relax into closed circles and then assemble nucleosomes. When the DNA is purified, the process of chromatin assembly is observed as a gradual upward
shift in a ladder of negatively supercoiled topoisomers resolved on a chloroquine agarose gel (one negative supercoil for each nucleosome) (Fig. 1, lane D). When the cytoplasm enters the S phase of the first cell cycle about 27 min after calcium ionophore activation (11), preassembled FV1 molecules have more nucleosomes than do FV1 molecules injected into eggs 10 min after activation (Fig. 1, lanes E and F). Observations like this led us to postulate that preassembled FV1 molecules replicate more efficiently because they have a head start on the pathway leading to replication in the first cell cycle (6).

The Preloading Effect in Vitro—Preincubation of FV1 DNA in a CSF extract prepared from unactivated eggs also results in efficient plasmid replication once the extract is activated by addition of CaCl$_2$ (Table I). 2 h of preincubation in CSF extracts results in replication of 30–40% of the input FV1 DNA, an efficiency comparable to that observed in ovum (6). However, the kinetics of in vitro replication are somewhat slower (Fig. 2).

In this experiment, we prepared FV1 chromatin with increasing numbers of nucleosomes/molecule by incubating the plasmid in a high speed supernatant prepared from activated eggs (18) for up to 570 min (Fig. 3). DpnI digestion of each of these DNAs (data not shown) revealed that none had replicated. This was expected because the high speed activated extract lacks the membrane vesicles required for pseudonucleosome formation (3, 19, 20). Next, we diluted each of the preassembled chromatin templates into a CSF extract either ten minutes before or immediately after addition of calcium. According to our original hypothesis, we expected preassembled chromatin templates to replicate efficiently in activated extracts, without need of any additional exposure to the unactivated CSF cytoplasm. This was not the case.

Even the template assembled into chromatin for 570 min replicated poorly when added directly to the calcium-treated CSF extract (Fig. 3B). The same was also true for templates with fewer nucleosomes/molecule (data not shown). In contrast, incubation of these same chromatin templates in unactivated CSF extract for 10 min prior to addition of calcium resulted in efficient replication (Fig. 3B). These results clearly demonstrate that prior chromatin assembly per se does not account for the preloading effect.

Chromatin Assembly and the Preloading Effect in Vitro—Further doubts about the importance of prior chromatin assembly came from chloroquine gel analysis of FV1 molecules that had and had not been incubated in CSF extract for 2 h before activation of the cell cycle. Because preincubated molecules replicated much more efficiently (Fig. 2), we expected them to be more highly supercoiled at the very start of the in vitro cell cycle, as observed in eggs (Fig. 1, right panel). However, this was not the case. In fact, both preincubated and nonpreincubated plasmids display the same level of chromatin assembly at equivalent times after activation (data not shown, but see Fig. 4A). We conclude that chromatin assembly does not take place prior to the addition of calcium and therefore cannot account for efficient plasmid replication after activation.

Replication of Plasmid Chromatin Templates in Vitro—The above conclusion was independently confirmed by examining the replication of preassembled chromatin templates in vitro. In this experiment, we prepared FV1 chromatin with increasing numbers of nucleosomes/molecule by incubating the plasmid in a high speed supernatant prepared from activated eggs (18) for up to 570 min (Fig. 3A). DpnI digestion of each of these DNAs (data not shown) revealed that none had replicated. This was expected because the high speed activated extract lacks the membrane vesicles required for pseudonucleosome formation (3, 19, 20). Next, we diluted each of the preassembled chromatin templates into a CSF extract either ten minutes before or immediately after addition of calcium. According to our original hypothesis, we expected preassembled chromatin templates to replicate efficiently in activated extracts, without need of any additional exposure to the unactivated CSF cytoplasm. This was not the case.

Even the template assembled into chromatin for 570 min replicated poorly when added directly to the calcium-treated CSF extract (Fig. 3B). The same was also true for templates with fewer nucleosomes/molecule (data not shown). In contrast, incubation of these same chromatin templates in unactivated CSF extract for 10 min prior to addition of calcium resulted in efficient replication (Fig. 3B). These results clearly demonstrate that prior chromatin assembly per se does not account for the preloading effect.

**Table I. The preloading effect in vitro: preincubation of FV1 DNA in CSF extracts increases replication efficiency following extract activation**

| Time of preincubation in CSF extract | Replicated molecules at 240 min after activation |
|-------------------------------------|-------------------------------------------------|
| None                                | 6                                               |
| 1 min                               | 19                                              |
| 90 min                              | 27                                              |
| 120 min                             | 36                                              |

**Fig. 2. The preloading effect in vitro: kinetics of FV1 DNA replication.** FV1 DNA (final concentration, 4 ng/μl) was preincubated for 120 min in a freshly prepared CSF extract. The extract was then activated and sampled over time for replicated molecules. An additional aliquot of FV1 DNA was added directly to a CSF extract 10 min after activation and was assayed for replication. The results show that preincubated DNA replicates efficiently and over a long period of time, whereas DNA that is not preincubated hardly replicates at all. The percentage of replicated molecules corresponds to the fraction of each sample that is resistant to digestion with DpnI.
formed in ovum, albeit as a much smaller fraction of the total sample (see Fig. 1, lane D). Given these facts, we sought to identify this unique FV1 conformation and to investigate its possible contribution to the preloading effect.

Two-dimensional chloroquine gel analysis (16) established that the new forms of FV1 recovered from CSF extracts correspond to nicked knotted plasmids (Fig. 4B). Whereas a standard set of negatively supercoiled molecules forms an arc of discrete spots in these two-dimensional gels, the ladder of FV1 molecules recovered from CSF extracts migrates in a straight diagonal line, i.e. independently of chloroquine concentration.

This pattern of migration is characteristic of a family of nicked knotted DNA circles formed when plasmid DNA is exposed to high concentrations of topoisomerase II in vitro (13–15). The most prominent band below the nick circles is the trefoil or pretzel form and has only a single knot, whereas the more rapidly migrating but less abundant forms have increasing numbers of knots (13). DNA knotting in egg extracts is probably due to the large stockpiles of topoisomerase II present in Xenopus eggs (21).

The experiments shown in Fig. 5 confirmed that plasmid DNA recovered from CSF extracts is indeed knotted. DNA
knotted requires a high ratio of active topoisomerase II to plasmid DNA molecules (12-14). Accordingly, increasing plasmid DNA concentration in the CSF extract or lowering topoisomerase II activity by supplementing the extract with phosphatase inhibitor β-glycerol-P0₄ inhibited DNA knotting to different extents (Fig. 5). Interestingly, the same conditions that prevent DNA knotting also allow generation of a ladder of FV1 topoisomers characteristic of chromatin templates (Fig. 5). We conclude that rapid plasmid DNA knotting hinders chromatin assembly in the CSF extract.

DNA Knotting in CSF Extracts Does Not Account for the Preloading Effect in Vitro—Although extensive, DNA knotting observed in unactivated CSF extracts is unlikely to account for efficient plasmid replication after activation, because high levels of DNA knotting are not observed in unactivated whole eggs injected with FV1 (see Fig. 1, lane D). Nevertheless, we could not rule out a role for knotting in vitro because β-glycerol-P0₄, which suppresses knotting in vitro (Fig. 5), also inhibits DNA synthesis (data not shown).

In order to make sure that knotting does not play a role in the preloading effect, we directly compared the replication of purified, non-knotted and supercoiled FV1 molecules added directly to freshly activated CSF extracts. As shown in Fig. 6A, nicking of knotted plasmids was prevented by addition of 0.5 M NaCl to samples prior to DNA isolation (see Ref. 14 and “Materials and Methods”). Both knotted and supercoiled molecules replicated poorly in activated CSF extract (Fig. 6B). In contrast, control samples of supercoiled FV1 DNA added to CSF extracts before activation once again replicated efficiently, proving that the extract was fully competent for replication. We conclude that knotting per se does not promote efficient plasmid replication and consequently does not account for the preloading effect.

**DISCUSSION**

This paper describes a new experimental system for the efficient replication of small circular plasmid DNAs in extracts prepared from unfertilized Xenopus eggs. Incubation of plasmid molecules in metaphase arrested extracts, like unactivated whole eggs, enhances their subsequent replication when the cytoplasm or intact egg is activated to re-enter the cell cycle. In contrast, the same DNA added directly to an already activated cytoplasm, or egg, replicates poorly. Efficient plasmid replication takes place in freshly prepared CSF extracts but has thus far failed in frozen and thawed CSF extracts activated by addition of calcium. This is probably because unlike fresh extracts, frozen and thawed CSF extracts can only be fully activated by diluting them into a second extract prepared from activated eggs (11).

Even though our in vitro system duplicates the preloading effect, our analysis of chromatin assembly in vitro does not support our earlier conclusion from intact eggs that efficient replication depends on chromatin assembly before the start of the cell cycle (6). For instance, although FV1 is rapidly knotted in CSF extract and does not assemble into chromatin, it nevertheless replicates efficiently after activation. Conversely, preassembled chromatin templates do not replicate efficiently in activated egg extracts unless these templates are briefly exposed to an unactivated CSF extract. We conclude that efficient replication depends on factors or enzymatic activities present in CSF extract that are distinct from those required for chromatin assembly or knotting.

In view of the findings reported here, how can we explain our results using intact eggs, in which both the timing and the amount of replication appeared to quantitatively correlate with the extent of prior chromatin assembly (6)? It has been clearly established that plasmid replication in Xenopus eggs and ex-
treats requires previous chromatin assembly, formation of a pseudonucleus, and assembly and activation of DNA replication centers (2, 3). Thus, the correlation between chromatin assembly and efficient replication in ovum is probably not fortuitous. However, chromatin assembly in unactivated eggs may mask the fact that other factors of the metaphase cytoplasm also bind to FV1 DNA and play a critical role in subsequent template replication.

In the course of these studies we also discovered that CSF extracts can introduce topological knots into plasmid DNA due to the high levels of topoisomerase II activity in these extracts. DNA knotting appears to explain the failure of CSF extracts to assemble plasmid chromatin, but we cannot rule out the possibility that additional replication-enhancing proteins bind to knotted FV1 DNA. Our findings demonstrate that CSF extracts prepared under standard conditions differ in important respects from the cytoplasm of intact unactivated eggs. Conditions that prevent DNA knotting in vitro favor chromatin assembly, which predominates in ovum. The fact that β-glycerophosphate, a phosphatase inhibitor, enhances chromatin assembly in vitro suggests that the ratio of kinases to phosphatase is higher in intact eggs than in extracts prepared under standard conditions. The effect is probably indirect, via regulation of topoisomerase II activity in the extract. Hyperphosphorylation of topoisomerase II is known to decrease its affinity for DNA (22).

Finally, the fact that CSF extract enhances replication of preassembled chromatin makes it possible for the first time to distinguish between chromatin assembly per se and the biochemical changes in template structure required for replication. We now predict that the CSF determinants responsible for enhancing subsequent replication must 1) interact with both naked DNA and with DNA already assembled into chromatin, 2) bind to these substrates very rapidly, 3) disappear quickly from CSF extract upon addition of calcium, and 4) reappear in activated eggs when they progress into first mitosis.

The above characteristics of our replication-enhancing determinant are compatible with “replication licensing factor”, an activity controlling initiation of nuclear DNA replication in Xenopus egg extracts (7). Replication licensing factor is thought to gain access to the DNA during mitosis when nuclear envelope breakdown occurs and is believed to become active upon exit from metaphase (23–25). In this regard, the preloading effect could be viewed as the result of mitotic egg cytoplasm licensing plasmid DNA for efficient replication. Accordingly, the Xenopus homologues of the yeast MCM3 and mammalian P1 family recently identified as components of licensing factor in frog egg extracts (8–10) become likely candidates for the preloading factor. We are currently investigating the relationship between licensing factor and preloading factor by testing the replication efficiency of plasmid DNA assembled into chromatin in vitro in the presence or the absence of MCM-3 protein in activated extracts devoid of licensing factor activity (23). The in vitro system and experimental conditions described here will permit characterization of replication-enhancing activities in mitotic egg cytoplasm in the context of a small, well characterized plasmid substrate rather than in the context of the complex genome of whole eukaryotic nuclei.

Acknowledgments—We thank Dr. James Wang for his insights into DNA knotting.

REFERENCES
1. Almouzni, G., and Wolffe, A. P. (1993) Exp. Cell Res. 205, 1–15
2. Newport, J. (1987) Cell 48, 205–217
3. Blow, J. J., and Sleeman, A. M. (1990) J. Cell Biol. 95, 383–391
4. Marini, N. J., and Benbow, R. M. (1991) Mol. Cell. Biol. 11, 299–308
5. Wangh, L. J. (1989) J. Cell Sci. 93, 1–8
6. Sanchez, J. A., Marek, D., and Wangh, L. J. (1992) J. Cell Sci. 103, 907–918
7. Blow, J. J., and Laskey, R. A. (1988) Nature 332, 546–548
8. Kubota, Y., Mimura, S., Nishimoto, S., Takisawa, H., and Nojima, H. (1995) Cell 81, 601–609
9. Chong, J. P. J., Mahabubani, H. M., Chong-Yee, K., and Blow, J. J. (1995) Nature 375, 418–421
10. Madine, M. A., Khoz, C. Y., Mills, A. D., and Laskey, R. A. (1995) Nature 375, 421–424
11. Wangh, L. J., Degrace, D., Sanchez, J. A., Yeghiazarians, Y., Wiedemann, K., and Daniels, S. (1995) J. Cell Sci. 108, 2187–2196
12. Liu, L. F., Rowe, T. C., Yang, L., Tewey, K. M., and Chen, G. L. (1983) J. Biol. Chem. 258, 15365–15370
13. Liu, L. F., Liu, C. C., and Alberts, B. M. (1980) Nature 281, 105–120
14. Hsieh, T. (1983) Mol. Cell. Biol. 3, 258, 418–421
15. Liu, L. F., Rowe, T. C., Yang, L., Tewey, K. M., and Chen, G. L. (1983) J. Biol. Chem. 258, 15365–15370
16. Wangh, L. J., Degrace, D., Sanchez, J. A., Gold, A., Yeghiazarians, Y., Wiedemann, K., and Daniels, S. (1995) J. Cell Sci. 108, 2187–2196
17. Blow, J. J., and Laskey, R. A. (1988) Nature 332, 546–548
18. Hsieh, T. (1983) J. Biol. Chem. 258, 8413–8420
19. Hsieh, T. (1983) J. Biol. Chem. 258, 8413–8420
20. Rocas, B., Berger, J. M., and Wang, J. C. (1993) J. Biol. Chem. 268, 14250–14255
21. Blow, J. J. (1993) J. Biol. Chem. 268, 5975–5979
22. Vasseztzky, Y. S., Dang, Q., Benedetti, P., and Gasser, S. M. (1994) Cell 81, 601–609
23. Blow, J. J. (1993) J. Biol. Chem. 268, 5975–5979
24. Kubota, Y., and Takisawa, H. (1993) J. Cell Biol. 122, 993–1002
25. Coverley, D., Downes, C. S., Romanowski, P., and Laskey R. A. (1993) J. Cell Biol. 122, 985–992
