Chromatin Aggregation Changes Substantially as pH Varies within the Physiological Range*

(Rceived for publication, March 13, 1989)

Xiao-wen Guo and R. David Cole
From the Department of Biochemistry, University of California, Berkeley, California 94720

In vitro at least, changes of pH within the physiological range are important in regulating chromatin aggregation. The extent of aggregation was shown to depend substantially on pH as well as on salt. In the absence of salt and in all of the salt conditions tested as the pH was increased, less and less chromatin was aggregated. The aggregation of half the chromatin in the presence of 0 mM NaCl, 150 mM NaCl, 250 mM NaCl and 1 mM MgCl2, and 2 mM MgCl2 was observed at pH 5.4, 6.0, 7.0, and 8.3, respectively. In 150 mM NaCl, 1 mM MgCl2, chromatin aggregation decreased from 86 to 63% as the pH was changed from 5.9 to 8.8, which is the same pH change reported to occur between interphase and mitosis. H1 dissociation from chromatin was also found to be pH dependent.

In vitro, strands of chromatin can undergo linear compaction, higher order folding, and gross aggregation in the presence of various amounts of salt. At low levels of salt a 10-nm thick beads-on-a-string structure is the dominant feature of chromatin, as detected by electron micrographs (1). As the concentration of salt is increased to about 30 mM, compaction of the 10-nm fiber occurs, and as the salt concentration is increased further, the 30-nm structure (solenoid) forms (2, 3). X-ray diffraction studies show a 10-nm reflection which is taken to be the repeat distance from one 10-nm filament to the next along the axis of the 30-nm fibers (4). In addition, sedimentation results of chromatin from rat liver or chicken erythrocytes can be interpreted in terms of the gradual folding of the 10-nm fiber to the 30-nm fiber structure (5, 6) as the salt concentration was raised from 40 to 60 mM. A further increase in salt concentration to isotonic concentrations generates the aggregation and precipitation of chromatin (7, 8). As salt concentrations are still further increased, chromatin is resolubilized and eventually histone H1 dissociates (7, 8).

Chromatin aggregation can be induced by monovalent cations such as Na+, as well as by Mg++ and other multivalent cations (7-10). At Na+ and Mg++ concentrations resembling physiological conditions, precipitation of the chromatin is partial and there appears to be an aggregation-prone class and an aggregation-resistant class of chromatin (8). H1 histone is nonuniformly distributed between these classes and there are restrictions on the exchange of H1 between these classes of chromatin (8, 10-12). The aggregation-prone class of chromatin might well be analogous to the clumps of transcriptionally inactive heterochromatin revealed by electron micrographs of sectioned nuclei (13, 14). Chromatin fractionation schemes aimed at the separation of heterochromatic domains from uncondensed domains would of course depend on the maintenance of the native degree of chromatin condensation. The sensitivity of chromatin fractionation schemes to modest variations of salt concentration in the physiological range has therefore increased our concern to approach physiological conditions as closely as reasonable in such experiments. Since the physiological state is not precisely defined, however, it seemed worthwhile to determine the likely range of variation in chromatin condensation produced by alterations of solution conditions within the physiological range. To this end we recently examined chromatin condensation in solutions containing monovalent and multivalent cations, singly and in combination (10).

Since the components of chromatin include highly negatively charged DNA and hily positively charged histones, the effects of cations on chromatin condensation and aggregation can be rationalized partially as due to a masking of negative charges by the cations, and a competition between simple cations and H1 histone for binding sites on the DNA. Perhaps similar effects might result from titration of charges by H+ or OH-. To find out whether or not significant alterations of chromatin condensation actually occur over the physiological range of pH, we set forth to study systematically the role of pH in salt-induced chromatin aggregation, and in the binding of H1 to chromatin.

MATERIALS AND METHODS

Preparation of Nuclei—All steps in sample preparation were performed at 0-4 °C. Cell culture and nuclear isolation were as described previously (10). HeLa cells were labeled for 24 h with the addition of 0.25-0.5 μCi/ml [3H]thymidine to the growth medium. Pelleted HeLa cells were washed with phosphate-buffered saline solution once before isolation of their nuclei. Finally, the nuclei were washed and stored in buffer A (10 mM Tris·HCl, 90 mM NaCl, 2 mM MgCl2, 0.34 M sucrose, 1.0 mM phenylmethylsulfonyl fluoride, pH 7.5) or in buffer B (50 mM Tris·HCl, 25 mM KCl, 5 mM MgCl2, 0.34 M sucrose, 1.0 mM phenylmethylsulfonyl fluoride, pH 7.5).

Preparation of Chromatin—Nuclei in buffer A or buffer B were adjusted to A260 = 50 and incubated at 37 °C for 5 min, before a mixture of CaCl2 (1 mM) and micrococcal nuclease was added (50 units/ml for nuclei in buffer A for preparation 1, and 20 units/ml for nuclei in buffer B for preparation 2). After incubation (3 min for preparation 1, 6 min for preparation 2 and 3 min for digestion), digestion was stopped by adding EGTA' (1 mM). All subsequent steps were at 0-5 °C. After digestion nuclei were collected by centrifugation and then lysed and extracted twice (2 h each) with equal volumes of 10 mM Tris, 2 mM EDTA (pH 7.5), on ice with occasional agitation. The chromatin released was pooled and dialyzed extensively against 100 volumes of 5.0 mM Tris, 0.1 mM EDTA (pH 8.0) for 48 h with two changes of the dialysis buffer. The chromatin solution was then centrifuged at 10,000 rpm in an SW 54 rotor for 5 min to remove any nuclear debris. Chromatin so prepared was stored at 4-5 °C and used within 3 days after nuclease digestion. The sizes of the chromatin fragments were analyzed by

* 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 1754 solely to indicate this fact.

1 The abbreviation used is: EGTA, [ethylenebis(oxyethylenem-}

nitrilo)tetraacetic acid.
extracting DNA from nuclear fractions and submitting them to electrophoresis on agarose gel (10).

The Dependence of Salt-induced Chromatin Aggregation upon pH—Chromatin solutions were adjusted to the desired pH by addition of small aliquots of NaOH or HCl (0.1 to 1.0 n) at 20 °C. Salt solutions used for pH dependence experiments were also adjusted to the appropriate pH before use. Chromatin and salt were mixed to a final chromatin concentration of \( A_{260} = 6 \) at the desired pH and concentration of salt; the mixture was then incubated at 20 °C for 2 h. Aggregated chromatin (pellet) and nonaggregated chromatin (supernatant) were then separated by microfuge sedimentation for 2 min. Longer centrifugation (up to 8 min) of the mixture did not increase the yield of aggregated chromatin (the pellet). The solubility of chromatin was calculated as the percentage of the total starting material (\( A_{260} \)) or radioactivity that remained in the supernatant. The measurement of solubility was the same whether \( A_{260} \) or radioactivity was used.

Circular Dichroism Analysis of Soluble Chromatin—Soluble chromatin released from nuclei was adjusted to \( A_{260} = 1.0 \) in 5 mM NaPO4, 0.2 mM EDTA (pH 7.0) buffer. The sample was degassed and pH adjusted with 0.1 N NaOH or HCl. The circular dichroism spectra were taken at 23 °C in a 1-cm path cell on a circular dichroism spectrometer, Aviv model 60DS.

Histone H1 Dissociation and Protein Analysis—Mixtures of chromatin and salt at the desired pH and salt concentration were incubated at 20 °C for 2 h before subjecting them to ultracentrifugation (100,000 X g, 4 °C, 20 h). The relative concentrations of protein in the supernatant and pellet were measured after electrophoresis through a 14.5% polyacrylamide gel containing 0.1% sodium dodecyl sulfate (15). The protein content was estimated by scanning six to eight Coomasie Blue-stained gels in an Ultrascan Laser Densitometer Model 2202 (Pharmacia LKB Biotechnology Inc.).

RESULTS

The Dependence of Chromatin Aggregation upon pH—The effect of pH on salt-induced chromatin aggregation was measured by precipitation of the chromatin as a function of pH in the presence of various salts. In the absence of salt, chromatin showed (Fig. 1) a sharp transition in solubility at pH 5.4, and below this pH complete precipitation of chromatin occurred. Similarly, when the salt concentrations were 150 mM NaCl or 150 mM NaCl and 1 mM MgCl2, or 2 mM MgCl2 alone, aggregation of chromatin was highly dependent on the pH of the solution (Fig. 1) but the position of the sharp transition differed from one salt condition to the next. In the pH range tested, the ability of salt to induce aggregation of chromatin decreased as the pH increased and such an effect was particularly abrupt between pH 5.5 and 8.5. It was striking that at pH 7.5 only 14% of chromatin was aggregated in the presence of 150 mM NaCl, and 50% in the presence of both 150 mM NaCl and 2 mM MgCl2 as summarized in Table I. In these experiments the size of the chromatin fragments did not play a dominant role in the determination of solubilities as shown in Fig. 2. Two preparations of chromatin (Fig. 2A) were subjected to salt-induced chromatin aggregation experiments: preparation 1 was a pool of two successive extractions (see lanes 1 and 2) of nuclei digested 5 min in buffer A; preparation 2 was a pool of extractions (lanes 3 and 4) of nuclei digested 3 min in buffer B. The majority of fragments in preparation 1 contained 6 or fewer nucleosomes and the fragments in the other preparation were mostly from 6 to 50 nucleosomes. The two chromatin preparations gave similar pH-dependent aggregation profiles (Fig. 2B). We conclude therefore that the

![Fig. 1](image1.png)

**FIG. 1.** pH dependence of salt-induced chromatin aggregation. Chromatin (\( A_{260} = 6.0 \)) at the desired pH and salt concentration were incubated at room temperature for 2 h. Soluble chromatin in the presence of 0 mM NaCl (●), 150 mM NaCl (○), 150 mM NaCl and 1 mM MgCl2 (+), or 2 mM MgCl2 (Δ) was then separated from aggregated chromatin by microfuge (2 min). All \( A_{260} \) readings were taken in 0.1 N NaOH.

![Fig. 2](image2.png)

**FIG. 2.** The pH dependence of chromatin aggregation is not affected by the size of the chromatin. Panel A, DNA purified from soluble chromatin fractions of either preparation 1 (lanes 1 and 2) or preparation 2 (lanes 3 and 4) was subjected to 1.2% agarose gel electrophoresis. Lane M contained size markers of X DNA digested with HindIII. The fragment sizes are marked as kilobase pairs (KB). Panel B, chromatin fractions as shown in panel A. Lanes 1 and 2 (open symbols) or lanes 3 and 4 (solid symbols) were pooled for this aggregation experiment. Solution conditions: 150 mM NaCl (□ ■); 150 mM NaCl, 1 mM MgCl2 (Δ, ▲).

**TABLE I**

| Concentration | pH at 1/4 ppt | Extent of ppt |
|---------------|--------------|--------------|
| 0 0 | 5.4 | 5 0 |
| 150 0 | 6.0 | 33 14 |
| 150 1 | 7.0 | 73 30 |
| 150 2 | 7.9 | 79 50 |
| 0 2 | 8.6 | 88 70 |
pH dependence of chromatin aggregation is an inherent feature of chromatin.

As shown previously (8) when the NaCl concentration was increased gradually from 0 to 500 mM, maximal aggregation was obtained at 150 mM NaCl; aggregation increased as salt concentration was changed from 0 to 150 mM and then decreased as the NaCl concentration was raised further. The effect of pH on the maximal extent of aggregation can be gauged in Fig. 3, where solubilities of chromatin at pH 6, 7, or 8 were plotted as a function of salt concentration. Consistent with the data in Fig. 1, maximal aggregations at pH 6.0, 7.0, or 8.0 were 65, 30, and 13%, respectively.

That more subtle aspects of chromatin condensation than gross aggregation are affected by pH was shown by the use of circular dichroism. Because circular dichroism measurements require optical clarity, it is used with chromatin only at low concentrations of salt where aggregates remain soluble if they occur at all. The ellipticity of DNA measured near 280 nm is lower when the DNA is incorporated into core particles (16) or chromatin (17) than it is when the DNA is free. Moreover, as core particles swell when the salt concentration is lowered from 11 mM NaCl to 1 mM the ellipticity rises (18), and parallel phenomena have been reported for chromatin (19).

To learn whether similar structural changes would result from variations in pH, we observed circular dichroism spectra for chromatin in 5 mM sodium phosphate, 0.2 mM EDTA at pH 6.5, 7.4, and 8.0. As may be seen in Fig. 4, changes in structure were revealed by circular dichroism although the changes were modest in the physiological range.

**Dependence of Histone H1 Dissociation upon pH**—The effect of pH on chromatin aggregation might be due to a pH dependence of H1 binding to the chromatin fiber, changes in H1-H1 interaction, or stability of the nucleosome. The electrostatic interaction of H1 with the chromatin fiber as a function of pH was investigated by testing the displacement of H1 with 300–600 mM NaCl. The displacement of H1 histone by NaCl is shown in Fig. 5. If there is an effect of pH on H1 release in this range of conditions it is evidently modest. Disassembly of nucleosomes in the presence of 600 mM NaCl was revealed at pH 6.0 and lower by the release of a partial complement of core histones into the supernatant as is evident in Fig. 5D; thus the nucleosome was not stable at pH below 6.0 in 600 mM NaCl. We conclude that H1 binding to the chromatin fiber is only modestly pH dependent in the physiological range.

**DISCUSSION**

Chromatin aggregation may result from partial neutralization of the charged groups of DNA by protons and from masking of charges by salts (20) as well as from bridging between chromatin fibers by the electrostatic interactions between DNA and histone H1 (10, 21). Therefore the aggregation of chromatin in vitro could depend on many solution conditions (10, 22), including the histone H1 content and concentration of chromatin fragments, the kinds and concentration of salts, the temperature, and as shown in this study, pH. Moreover, there is evidence (22) that hydrophobic forces contribute to chromatin aggregation in that both D2O and Triton X-100 reduce the extent of aggregation. Evidence for major factors, in addition to hydrophobic forces, is the observation that the yields of chromatin aggregation approached 90–100% in 100–200 mM NaCl at pH 7–8 when the temperature was 0–4 °C (9, 23) but only attained the level of 50 ± 20% at room temperature (8, 10, 24). Such complications in the mechanisms that regulate chromatin condensation make it difficult to interpret the simple data in Table I, but at least it is clear that pH plays one of the leading roles.

In addition to charge neutralization of DNA and interference with DNA-H1 interactions, low pH might increase chromatin aggregation by its effect on the nucleosomal core. Conformational transitions of core particles have been reported to be dependent on pH as well as on ionic strength (17), and while the relation of such transitions to the condensation of H1 histone-containing chromatin fragments is not clear, it is nonetheless intriguing that the transition of the core particle (25) as pH was lowered, particularly as measured with fluorescence, paralleled the change that was observed for chromatin solubility as presented in our study. The correlation could suggest that protonation of the core particle might be one factor leading chromatin to become more highly aggregated at pH values below the transition point. The structural transitions of core particles at low pH are perhaps related to the dissociation of histone octamers observed in other studies (26). The changes in circular dichroism presented in Fig. 4,
which are comparable to those of core particles in the same pH range (25), could have their basis in structural transitions of the nucleosome as well as in fiber condensation. It is unfortunate that increasing turbidity prohibits the use of circular dichroism below pH 6 where a correlation with the core particle studies might have been tested more extensively.

Unfortunately, in structural studies of chromatin, little consideration has been given to the possible effects of pH on the state of chromatin, probably because the physiological range of pH (about 7.0–7.5) (27) is far removed from the isoelectric points of the histones (>10) and DNA (<2.5). The lack of concern for possible effects of pH on chromatin structure can be illustrated by the fact that the structure of chromatin has been studied by physical methods at pH 7.0–7.8 (5, 28, 29), by chemical cross-linking at pH 5.5 (30, 31), 7.0 (32), 8.5 (30, 31), 8–9 (33–35), and indefinite pH (35). Similarly attempts to fractionate chromatin by nuclease treatments used pH values from 6.5 to 8 (8–10, 24). A change in conformation of H1 histone in the physiological range of pH (37) could have profound effects on the condensation of chromatin since the latter is completely dependent upon H1. In fact, although the experiments went well outside the physiological range (to pH 10), Losa et al. (38) (see also Ref. 39) demonstrated that the folding of chromatin into a 30-nm fiber was prevented when the pH was raised even though H1 remained bound to chromatin; this effect of high pH was attributed to changes in H1-H1 interactions, possibly involving their globular domains. Clearly, then, variations in pH could lead to differences in the outcome of the experiments on the physical structure of chromatin and the interpretation of the data.

Chemical cross-linking studies of chromatin have been done over a broad range of pH values, from as low as 5.5 to as high as 9.0 (30–36). Chemical cross-linking reactions were done at pH values other than neutral to increase the cross-linking efficiency. In choosing such pH conditions one should be aware of the possible artifacts introduced by either acidic or alkaline pH. For instance, to test for changes in the nearest neighbors of H1 histone as chromatin condensed from the 10-nm fiber to the 30-nm fiber, the chromatin was treated with cross-linkers at 0–80 mM NaCl. No appreciable salt sensitivity was observed in the cross-linking pattern of H1 and it was concluded that histone H1-H1 interactions were not affected by chromatin condensation (34). It now becomes clear, however, that since the cross-linking studies were performed at pH 8–9, the lack of ionic strength dependence of H1 polymer formation could be explained by the fact that in this pH range the majority of the chromatin was not condensed even in the presence of salt (Fig. 3) and probably existed in its extended form. In support of this possibility, it has been reported that α-chymotryptic digestion of chromatin in the presence of 100 mM NaCl, revealed protection of the COOH-terminal domain of H1 at pH 7 but showed its exposure at pH 9, thus indicating a conformational difference in the chromatin structures (38) at the two pH values tested. Electron microscopic studies of chromatin structure also showed that at higher pH (9–10) the 30-nm fiber could not be formed even though H1 remained bound to chromatin (39). This observation is consistent with our aggregation data.

Our own previous work (30) with a zero length cross-linker showed that relative yields of H1-H1 dimers were consistent with random combinations of the H1 variants. This could be taken to argue against the notion that particular H1 variants are enriched in particular domains of chromatin. The cross-linking was done, however, by incubation of nuclei with a carbodiimide at pH 5.5, and as a result of the present study

**Fig. 5. H1 displacement by NaCl.** Chromatin was incubated in the presence of 300–600 mM NaCl at pH 6.0, 7.0, or 8.0 for 2 h at room temperature before centrifugation at 100,000 × g, for 20 h at 4 °C. Equal amounts of the supernatant were analyzed by electrophoresis in 14.5% polyacrylamide, 0.1% sodium dodecyl sulfate gel and staining with Coomassie Blue. The amount of H1a (C) and H1b (B) released from the chromatin was measured by densitometry and plotted in arbitrary units of stain intensity for pH 6 (panel A), pH 7 (panel B), and pH 8 (panel C), respectively. An example of the gel electrophoresis is shown in panel D. The salt concentrations were: lanes 1, 6, and 11, 300 mM; lanes 2, 7, and 12, 350 mM; lanes 3, 8, and 13, 400 mM; lanes 4, 9, and 14, 500 mM; lanes 5, 10, and 15, 600 mM; lane 16 is the starting material.
we now understand that such a pH might have forced chromatin aggregation substantially beyond the native state, thus invalidating the conclusion that was drawn.

The disturbing conclusion of the present study combined with previous work is that within the range of uncertainty in our knowledge of physiological conditions, the state of aggregation of chromatin could vary substantially and that in vitro observations of higher order structure in isolated chromatin, or even in intact nuclei might not represent the native structure. Added to the uncertainty about the true physiological condition is the unavoidable use of nonphysiological conditions of salts, detergents, and pH for isolation of nuclei from cells or for isolation of chromatin from nuclei. It is not to be taken for granted that once native chromatin has been decondensed (for example, in dilute EDTA) it will refold into its original state when physiological conditions are restored. It may have some physiological significance. Although other factors are probably involved as well, the possible role of pH cannot be ignored.

In addition to revealing the vulnerability of in vitro structural studies of chromatin to artifact, the present observations may have some physiological significance. Although other factors are probably involved as well, the possible role of pH changes during the cell cycle with regard to their effects on the condensation state of chromatin in vivo cannot be ignored. The strong dependence of chromatin aggregation on pH from 5.5 to 8.5 in the presence of 150 mM NaCl with or without 2 mM MgCl₂ shows how variations of pH regulated in vivo could have physiologically significant effects on the conformation of chromatin. Some cellular processes, for example, mitosis, have been shown to be triggered by changes in pH as well as by changes in [Ca²⁺] concentration. Oscillations of pH in Physarum between 5.9 in interphase and 6.8 during mitosis have been reported by Gerson (27), who suggested that this rise in pH might stimulate DNA synthesis and division. The present results would be consistent with Gerson's suggestion since DNA synthesis might be stimulated by decondensation of chromatin occurring as a consequence of increased pH.

REFERENCES

1. Olins, A. L., and Olins, D. E. (1974) Science 183, 330-332
2. Finch, J. T., and Klug, A. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 1897-1901
3. Thoma, F., Koller, T. H., and Klug, A. (1979) J. Cell Biol. 83, 403-427
4. Speirling, L., and Klug, A. (1977) J. Mol. Biol. 112, 253-265
5. Butler, P. J. G., and Thomas, J. O. (1980) J. Mol. Biol. 140, 505-529
6. Thomas, J. O., and Butler, P. J. G. (1980) J. Mol. Biol. 144, 89-93
7. Strätling, W. H. (1979) Biochemistry 18, 596-603
8. Huang, H.-C., and Cole, R. D. (1984) J. Biol. Chem. 259, 14237-14242
9. Borochov, N., Ausio, J., and Eisenberg, H. (1984) Nucleic Acids Res. 12, 3089-3096
10. Jin, Y., and Cole, R. D. (1986) J. Biol. Chem. 261, 15805-15812
11. Jin, Y., and Cole, R. D. (1985) FEBS Lett. 182, 455-458
12. Jin, Y., and Cole, R. D. (1986) J. Biol. Chem. 261, 3420-3427
13. Gerace, L. (1986) Trends Biochem. Sci. 11, 443-446
14. Fakan, S., and Nobis, P. (1978) Exp. Cell Res. 113, 327-337
15. Laemmli, U. K. (1970) Nature 227, 680-685
16. Fasman, G. D. (1979) in Chromatin Structure and Function (Nicolini, C. A., ed) pp. 67-107, Plenum Publishing Corp., New York
17. Shih, T. Y., and Lake, R. S. (1972) Biochemistry 11, 4811-4817
18. Gordon, V. C., Schumaker, V. N., Olins, D. E., Knodler, C. M., and Howitz, J. (1979) Nucleic Acids Res. 6, 3845-3858
19. Regez, P. R., Weisman, D., Huvos, P. E., and Fasman, G. D. (1982) Biochemistry 21, 993-1002
20. Manning, G. S. (1978) Q. Rev. Biophys. 11, 179-246
21. Ausio, J., Sasi, R., and Fasman, G. D. (1986) Biochemistry 15, 4233-4242
22. Huang, H.-C. (1984) Ph.D. Thesis, University of California at Berkeley
23. Matyasova, J., Skalka, J., and Cechova, M. (1980) Stud. Biophys. 78, 43-50
24. Ridsdale, J. A., and Davie, J. R. (1987) Biochemistry 26, 290-295
25. Libertini, L. J., and Small, E. W. (1982) Biochemistry 21, 3327-3334
26. Kawashima, S., and Iwashiri, K. (1982) J. Biochem. (Tokyo) 91, 959-966
27. Gerson, D. F. (1978) in Cell Cycle Regulation (Jeter, Jr., J. R., Cameron, R., Padilla, G. M., and Zimmerman, A. M., eds) pp. 105-131, Academic Press, Orlando, FL
28. McGhee, J. D., Nickol, J. M., Felsenfeld, G., and Ran, D. C. (1983) Cell 33, 831-841
29. Suka, P., Bradbury, E. M., and Baldwin, J. P. (1979) Eur. J. Biochem. 97, 593-602
30. Ring, D., and Cole, R. D. (1979) J. Biol. Chem. 254, 11688-11695
31. Ring, D., and Cole, R. D. (1983) J. Biol. Chem. 258, 15361-15364
32. Jackson, V., and Chalkley, R. (1987) Biochemistry 26, 2315-2325
33. Jackson, V. (1987) Biochemistry 26, 2315-2325
34. Itkes, A. V., Glotov, B. O., Nikolaev, L. G., Prem, S. R., and Severin, E. S. (1980) Nucleic Acids Res. 8, 507-527
35. Smerdon, M. J., and Isenberg, I. (1976) Biochemistry 15, 4233-4242
36. Leu, A., Thoma, F., and Koller, Th. (1984) J. Mol. Biol. 175, 529-551
37. Lobhart, P., Thoma, F., and Koller, Th. (1984) Eur. J. Biochem. 23, 121-134
38. Thomas, J. O., and Kornberg, R. D. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 2626-2630
39. Thomas, J. O., and Khabaza, A. J. A. (1980) Eur. J. Biochem. 112, 501-513
40. Jackson, V. (1987) Biochemistry 26, 2315-2325
41. Wu, P., and Khabaza, A. J. A. (1980) Eur. J. Biochem. 112, 501-513
42. Weintraub, H., and Groudine, H. (1976) Science 193, 848-858