The Condensation of Chromatin in Apoptotic Thymocytes Shows a Specific Structural Change*

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Chromatin condensation and DNA cleavage at internucleosomal sites have been recognized early as hallmarks of apoptosis, and it has been suggested that extensive DNA chain scission could directly result in the formation of dense chromatin bodies. Here we have shown that no causal relationship exists between DNA degradation and chromatin condensation in glucocorticoid-induced thymocyte apoptosis. The chromatin rearrangement occurred independent of as well as prior to DNA cleavage and involved a specific conformational change at the nucleosome level. In the early stages of the process, the core particles appeared to be tightly packed face-to-face in smooth 11-nm filaments that progressively folded to generate a closely woven network. The network finally collapsed, producing dense apoptotic bodies. Since trypsin digestion relaxed condensed chromatin and histone H4 underwent appreciable deacetylation in the apoptotic cell, we suggest that changes in the DNA-histone interactions represented a major modulating factor of condensation.

Although the term “apoptosis” was originally derived from the Greek to emphasize cytoplasmic and nuclear alterations peculiar to the process of programmed cell death (1), no attempt has been made thus far to search for the molecular events underlying these changes, particularly the collapse of the bulk of chromatin into dense domains. The reasons for this delay in the development of a fundamental approach are manifold. In the first place, the unique condensed appearance of the apoptotic nucleus is closely associated with the cleavage of chromatin at internucleosomal sites (2), a circumstance that supports the hypothesis of a causal relationship between extensive chromatin digestion and condensation (3). This early view has recently been challenged on the basis of more refined determinations of the chain length of the DNA isolated from apoptotic cells (4–6) but has long distracted from the search for the molecular mechanisms involved in the process of condensation. Moreover, since apoptosis plays a key regulatory role in several physiological and pathological processes, major efforts are currently being directed to the elucidation of the biochemical aspects and to the identification of the genes involved in the activation of the cell death program. The onset of chromatin condensation might direct the orderly turning off of genes required for the execution of metabolic suicide, therefore warranting a detailed structural characterization of apoptotic chromatin and a search for terminal modulating factors.

Bearing in mind the spatial distribution of interphase chromatin, the appearance of the apoptotic nucleus immediately suggests the occurrence of a structural change involving extremely large domains, and the question arises whether a specific conformational transition at the nucleosome level might account for such a catastrophic phenomenon. In the first place, is chromatin in apoptosis characterized by a three-dimensional array of nucleosomes different from that prevailing in the interphase 30-nm fiber? It has recently become apparent that the polynucleosomal chain can exhibit a marked polymorphism, depending on the prevailing mode of interaction among nucleosomes. Both edge-to-edge and face-to-face contacts arrayed in a fixed helical path are effective in the stabilization of the solenoidal structure (7), but the recent confirmation of the presence of marked irregularities in the conformation of the interphase fiber points to the existence of a variety of nucleosomal packing conformations (8). At physiological ionic strength, homogeneous reconstituted oligonucleosomes in the absence of H1 fold into a contacting 90° “zig-zag” helical structure (9). In a recent paper, we have shown that the nucleosomes interact weakly in the 30-nm fiber. The experimental value of the interaction free energy is as low in magnitude as −5 kcal/nucleosome mole (10), so that even limited biochemical modifications of the histone complement could result in a structural change. In this report we show that the basic conformational feature of chromatin in apoptosis is the tight face-to-face packaging of nucleosomes, which might be related to the increase in the amount of the unacylated forms of histones H3 and H4 occurring in the course of condensation.

EXPERIMENTAL PROCEDURES

Thymocyte Incubation and Characterization of the Status of Chromatin and DNA Fragmentation—Suspensions of thymocytes from suckling rats were treated with 10−8 M methylprednisolone sodium succinate (MPS)† for different time periods, following Wyllie et al. (11); the cells were incubated at a concentration of 5 × 107/ml. Nuclei were obtained by incubation of the pelleted cells in dissociation medium (DM) (75 mM NaCl, 24 mM Na2EDTA, 5 mM NaHSO3, and 1 mM phenylmethylsulfonyl fluoride, pH 7.8) containing 0.15% (v/v) Triton X-100, following the procedure already employed for calf thymus (12), in the digestion experiment nuclei were incubated at 37 °C for 3 h with 80 units/ml micrococcal nuclease (Sigma) according to Russo et al. (10). For electron microscopy of thin sections, control, glucocorticoid-treated cells, and

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* The abbreviations used are: MPS, methylprednisolone sodium succinate; DM, dissociation medium; DSC, differential scanning calorimetry; AUT, acetic acid/urea/Triton X-100 polyacrylamide gel electrophoresis.

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nuclei were pelleted at 150 × g for 10 min; fixation, embedding, and sectioning were performed as already reported (13). DNA was isolated by digestion of purified nuclei with proteinase K (Serva) in 10 mM Tris-HCl, 5 mM EDTA, 0.5% SDS, pH 8.0, followed by extraction with phenol and chloroform, and the chain length distribution of the fragments was analyzed on 1.5% agarose gels (14).

Calorimetry—Differential scanning calorimetry (DSC) experiments were performed as already reported (12, 13). Nuclei were isolated from control and from MPS-treated thymocytes as described above. The material was resuspended in DM and pelleted by centrifugation at 10,000 × g for 15 min; weighed amounts (from 30 to 50 mg) of nuclear pellet were then transferred into large volume (75-ml) calorimetric capsules. Measurements were performed with a Perkin-Elmer DSC7 (Perkin-Elmer Corp.) at a scan rate of 10 °C/min (12).

Electron Microscopy of Isolated Chromatin—Ten mg of pelleted nuclei were incubated in 10 ml of 0.1 mM Na2EDTA, pH 7.2, at 4 °C for 45 min; long incubation times (5 h) were required to completely unfold the condensed chromatin domains. In certain experiments, after a 45-min incubation chromatin was condensed by adjusting the concentration of NaCl with appropriate amounts of concentrated DM to 0.128 M. The nuclear suspensions were then centrifuged at 1,700 × g for 15 min, and chromatin was fixed by overnight dialysis of the supernatant at 4 °C against 0.1 mM Na2EDTA or DM containing 0.1% glutaraldehyde. The concentration of DNA was then adjusted to 3 mg/ml, and chromatin was mounted for electron microscopy on carbon-coated grids according to a standard protocol (15). The specimens were shadowed with platinum and observed in a Siemens 102 electron microscope operating at 80 kV.

Trypsin digestion and topoisomerase I treatment were carried out as follows. One mg of pelleted nuclei was resuspended in 1 ml of DM and digested with 100 µg/ml trypsin (type 8642, Sigma) at 25 °C for 15 min.
or 1 h as already reported (12). For reaction with topoisomerase I (TopoGEN) 40 mg of nuclear pellets were incubated in 0.5 ml of 100 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 10 mM β-mercaptoethanol, and 10 mM Tris-HCl, pH 7.5, containing 50 mg/ml bovine serum albumin in the presence of 500 units/ml enzyme for 3 h at 25 °C. Either preparation was processed for electron microscopy at low ionic strength as described above.

RESULTS AND DISCUSSION

To characterize the time course of apoptosis including early events, thymocytes from suckling rats were treated with MPS for different times (1, 4, 12, and 18 h) following the procedures of Wyllie et al. (11). The correlation between changes in the morphology of chromatin and DNA cleavage is shown in Fig. 1. The general trend conforms to previous observations (2, 3, 11, 20), although some new information has resulted. The DNA from cells treated for 1 h (Fig. 1B) does not migrate significantly through a 1.5% agarose gel, thus showing that the fragments have a chain length higher than 4 kilobase pairs (14); almost all of the cells have a markedly enlarged nucleus and a looser chromatin texture compared with the controls (Fig. 1A). After a 4-h exposure to MPS, the chromatin changes associated with apoptosis can be clearly recognized (Fig. 1C); at this time, the percentage of apoptotic thymocytes is ~18% as evaluated by isopyknic centrifugation on step Percoll gradients (20) (data not shown). Two distinct cellular subpopulations can be identified by electron microscopy. One corresponds to thymocytes that are clearly undergoing apoptosis; chromatin is packaged in dense masses beneath the nuclear envelope. The
other shows the same nuclear morphology (i.e. chromatin dispersion in thin punctuate bundles) as the thymocytes treated for 1 h. Centrifugation of the isolated DNA at 10,000 × g for 20 min in the buffer used for digestion with proteinase K prior to purification (see “Experimental Procedures”) yielded a pellet corresponding to more than 95% of the starting material in the form of very long fragments (lane 1 in Fig. 1C) and a supernatant (lane 2) containing the oligonucleosomal ladder, therefore affording biochemical evidence of the heterogeneity of the cell culture. Finally, after a 12-h exposure to MPS, an almost homogeneous population of apoptotic cells results; more than 95% of nuclei have changed into apoptotic bodies, while 40% of the DNA still possesses a chain length higher than 1.4 kilobase pairs (Fig. 1E). Only minor additional morphological changes were detected when the treatment was protracted for 18 h; nuclei underwent a somewhat more extensive fragmentation and there was a further decrease in the content of high molecular weight DNA (Fig. 1F).

While this brief re-examination of well established phenom-
eno logical features of apoptosis was needed to frame the new
observations reported here, it clearly leaves the problem of
the interrelation between DNA cleavage and chromatin condensa-
tion unsolved. Conventional electron microscopy of thin
nuclear section is capable of detecting gross rearrangements of
chromatin, but it fails to image the underlying structural de-
tails as a consequence of the poor penetration of the stain into
the embedding resin. The electron micrographs reported in Fig.
1, B and C, indicate that two successive morphological stages
are involved in the onset of apoptosis and further suggest that
the unfolding of the 30-nm fiber might represent an early event
but do not serve the purpose of ascertaining the occurrence of
changes in the spatial arrangement of nucleosomes. At the
same time, the lack of resolution of conventional agarose gel
electrophoresis beyond ~4 kilobase pairs (14) does not allow a
determination of the size distribution of long DNA fragments.
It has been reported that micrococcal nuclease digestion of
nuclei from normal thymocytes induces morphologic changes in
chromatin that mimic apoptotic condensation (3). We have
confirmed this result (Fig. 1G), but unfortunately micrococcal
nuclease is known to digest the RNA (21), and this process
elicits nuclear rearrangements superficially reminiscent of
those occurring in apoptosis (22). Recent reports using a vari-
ety of approaches suggest that alterations in chromatin may
occur in the absence of internucleosomal cleavage (4, 23, 24).
Only with the application of pulsed field gel electrophoresis to
the study of DNA chain length is it possible to reveal the
occurrence, at the earliest stages of the process, of long range
fragmentation at the level of the higher order structure or of
the loop (5, 6).

To establish in a straightforward way whether apoptosis
involves a structural change of chromatin relative to controls,
we carried out DSC determinations on nuclei isolated from
normal or MPS-treated thymocytes. The principles and ap-
lication of DSC technique are simple. For biological macromo-
cules, the measurements are normally carried out on solutions
or gels. The sample is transferred into high pressure calorimet-nic capsules, and its temperature is increased in the instrument
at a constant rate. When the denaturation temperature of the
macromolecular solute is reached, the unfolding process gives
rise to a peak in the heat absorption curve as a function of the
temperature. A typical thermogram consists of a series of heat
absorption peaks (or thermal transitions) with each peak cor-
responding to the unfolding of an energetically distinguishable
domain. Due to its sharp subdivision into two basic supramo-
lecular domains (the linker and the core particle) the poly-
nucleosomal chain lends itself to conformational studies by DSC.

Therefore, this technique is a powerful tool for investigating
the overall organization of chromatin in situ (10, 12, 13, 25–27). The
thermal denaturation profile of interphase nuclei shows two
major heat absorption peaks (labeled IV and V in Fig. 1H)
at 90 and 107 °C that arise from the melting of the core particle
DNA when the core particle is placed within an unfolded loop
and the higher order structure domains, respectively (12, 26).
Thus, the structural changes of nuclear chromatin can be quan-
titatively detected. By this approach, we and others have pre-
viously characterized the condensation process induced by salts
(12, 27) or histone H1 (10) in the absence of DNA cleavage as
well as the effect of endogenous or exogenous nucleases (26).
The DSC analysis of nuclear chromatin from thymocytes un-
dergoing apoptosis led to a clear-cut result (Fig. 1H). After a 1-h
exposure (scan 2), transition V (marked by arrows in scans 1–3,
5) at 107 °C (which is dominant in the thermogram of control
thymocytes (scan 1)) decreases, while the 90 °C endotherm
sharply increases; at this point the DNA is undegraded, while
electron microscopy shows diffuse nuclear changes (Fig. 1B).
Cells treated for 4 h show nothing but a shoulder at 103 °C
(scan 3) and a dominant endotherm at 90 °C despite the fact that ~95% of the DNA still has a high molecular weight (Fig.
1C). The fact that the calorimetric response characteristic of
interphase chromatin is lost early in the absence of appreciable
The level of the unacetylated isofrom of H4 increases during the induction of apoptosis. A (left-hand panel), AUT electrophoretic patterns of histones isolated from cells cultured for 4, 12, and 18 h in the absence (lanes 1–3) or presence (lanes 4–6) of 10^{-5} M MPS. 0, 1, and 2 indicate the unacetylated and mono- and diacetylated isofroms of H4, respectively (Coomassie Brilliant Blue-stained gels). Right-hand panel, the percentage of the unacetylated and mono- and diacetylated isofroms of H4 (triangles, circles, and squares, respectively) as a function of the time of incubation with filled symbols or without open symbols MPS. The points represent the mean ± S.D. of three to seven experiments. B–D, analysis of the incorporation of [3H]acetate. In three independent experiments, the thymocytes were cultured in the presence (○) or absence (●) of 10^{-5} M MPS for 2, 10, and 16 h; the radiolabel was then added to the medium (arrows in D), and the cells were collected for scintillation counting after 30, 60, and 120 min in the first and after 120 min in the second and third experiments, respectively. The dynamically acetylated sites are expressed as the percentage of the total number of modificable lysine sites. The points correspond to the mean ± S.E. of the values obtained from at least 10 scintillation countings from one to three separate preparations. The comparison between Coomassie staining (left-hand panels) and fluorography (right-hand panels) of the SDS-polyacrylamide gel electrophoresis gels of whole nuclear protein is shown in B and C. Note that the radiolabel is incorporated exclusively in histones H3 and H4. The electrophoretic analysis shown in B is relative to the first experiment reported in D. Lanes 1–3, control thymocytes cultured in medium alone; lanes 4–6, MPS-treated thymocytes collected 30, 60, and 120 min after the addition of [3H]acetate. The right-hand panel in C shows that when the radiolabel is added after 10 h of treatment the incorporation can no longer be detected by fluorography. Lanes 3 and 4, controls and thymocytes treated with MPS as in the second experiment reported in D. Lanes 1 and 2, control and MPS-treated thymocytes; [3H]acetate was added after a 2-h incubation with the steroid, and the cells were collected 2 h later.

Interucleosomal cleavage points to the occurrence of an overall structural rearrangement early in the induction of apoptosis.

The basic features of the structural change became apparent when chromatin was isolated by brief extraction at low ionic strength of nuclei from thymocytes for 12 or 18 h with MPS and fixed with glutaraldehyde to preserve the morphology present inside the cell. Neither filaments of nucleosomes nor the 30-nm solenoidal fiber could be observed by conventional electron microscopy of isolated chromatin (15). Instead, much of the material is organized in dense clumps surrounded by a network of smooth tortuous fibers 11 nm in diameter (Fig. 2B); often the network reduces to a halo of small loops (Figs. 2, C and D), suggesting that the central body corresponds to a nucleation center of an ongoing condensation process. Less condensed domains can also be found, confirming that successive steps are involved in the structural change. For example, the morphology of the sample shown in Fig. 2A suggests that the polynucleosomal chain forms very long 11-nm filaments before collapsing into dense bodies; it is apparent that the network represents an intermediate state arising from the folding of the filament on itself. Therefore, the basic structural difference between apoptotic chromatin and the “normal” counterpart mainly resides in the mode of interaction of nucleosomes; they appear to be tightly associated face-to-face, at variance with their radial disposition around the solenoid axis in the 30-nm fiber (7). This array is evident in high resolution micrographs. In certain regions, the orientation of the nucleosomes perpendicular to the carbon support film allows the visualization of the turns of DNA wrapped around the octamer (arrowheads in Fig. 2G).

We have characterized the perturbations induced in the structure of apoptotic chromatin by changes in ionic strength and by the selective digestion of core histones with trypsin. Prolonged exposure to a low salt buffer induces extensive unfolding of the domains, while the 11-nm filaments are not appreciably affected (Fig. 2E). Equilibration with DM (Na concentration, 0.128 M) restores the dense bodies (Fig. 2F). Thus, condensation in apoptosis is still driven by the screening of the electrostatic repulsion among phosphate groups, but the filaments are unable to undergo the transition to the 30-nm fiber. Trypsin digestion, which is known to degrade H1 and to detach the N-terminal tails of core histones (28), unravels the dense bodies and the fibrillar network (Fig. 3B) up to the extensive unfolding of the nucleosome (Fig. 3A). Very long stretches of DNA are frequently found; they are punctuated by dots, presumably due to the globular domains of histones that are still bound (arrows in Fig. 3A). Thus, as with interphase chromatin, the picture emerges that a major modulating factor of condensation resides in the interaction among the charged N-terminal domains and DNA. Since at physiological ionic strength the interaction free energy among nucleosomes in the 30-nm fiber is as low as a few kcal/nucleosome mole (10), even limited biochemical modifications might be sufficient to bring about the structural change.

We have investigated in some detail the time course of the level of core histone acetylation using AUT (17), which separates the acetylated isofroms of H4. We have found that the percentage of the unacetylated isofrom increases significantly during the induction of apoptosis, passing from 42.3 ± 0.4% in control thymocytes to 70.3 ± 5% in apoptotic cells (Fig. 4A). To inspect this result more closely, we have determined the percentage of lysine residues in core histones undergoing active acetylation-deacetylation by following the method of Zhang and Nelson (18). When a radiolabel ([3H]acetate) was added to the cell culture after a 2-h exposure to MPS and the sample was counted for radioactivity after 2 h, the percentage of dynami-
cally acetylated lysines was 4.23 ± 0.95% and 4.14 ± 0.50% for control and treated cells, respectively (Fig. 4D). When this protocol was applied to cells treated for 10 and 16 h, the incorporation dropped to 1.75 ± 0.07% and 0.53 ± 0.05%. These results taken together suggest that modulation of apoptosis relies at least in part on a decrease in the acetylation rate. The occurrence of a concomitant increase in the deacetylation rate cannot, of course, be ruled out on the basis of the present experiments.

The deacetylation of H4 can play two alternative functional roles. For viable cells, a great deal of evidence supports the hypothesis that highly acetylated core histones are preferentially associated with active or potentially active (competent) chromatin regions (29). Since it has to be expected that the cellular death program involves a progressive turning off of gene activity, H4 deacetylation might act as a general modulating factor of repression by supporting the compaction of the structure of transcribed genes. Alternatively, deacetylation might spread over the bulk of the chromatin, thereby triggering the massive structural change. The observed decrease in the acetylation of H4, if scattered throughout the genome, should increase the number of positive charges/nucleosome by 0.45 ± 0.10; the same figure is expected to arise from the deacetylation of H3 since these histones behave similarly in the incorporation experiments. The charge increase could in turn cause additional base pairs of DNA to interact with the histone core, thus forcing the central region of the linker to wind in a compensating right-handed toroidal supercoil. Leaving the detailed molecular interpretation out of consideration, it must be stressed that our experimental findings are consistent with the hypothesis that highly acetylated core histones are preferentially associated with active or potentially active (competent) chromatin regions (29). Since it has to be expected that the cellular death program involves a progressive turning off of gene activity, H4 deacetylation might act as a general modulating factor of repression by supporting the compaction of the structure of transcribed genes. Alternatively, deacetylation might spread over the bulk of the chromatin, thereby triggering the massive structural change. The observed decrease in the acetylation of H4, if scattered throughout the genome, should increase the number of positive charges/nucleosome by 0.45 ± 0.10; the same figure is expected to arise from the deacetylation of H3 since these histones behave similarly in the incorporation experiments. The charge increase could in turn cause additional base pairs of DNA to interact with the histone core, thus forcing the central region of the linker to wind in a compensating right-handed toroidal supercoil. Leaving the detailed molecular interpretation out of consideration, it must be stressed that our experimental findings are consistent with the hypothesis that an increase in the topological constraints of the linker directs the structural change. Therefore, relaxation of the supercoiled DNA domain should result in the re-establishment of the beads on a string configuration. The micrograph shown in Fig. 3C indeed supports this model, since the treatment of apoptotic chromatin with topoisomerase I abruptly converts the fibrillar chromatin into a field of well separated, connected nucleosomes.

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REFERENCES
1. Kerr, J. F. R., Wyllie, A. H., and Currie, A. R. (1972) Br. J. Cancer 26, 239–257
2. Wyllie, A. H. (1980) Nature 284, 555–556
3. Arends, M. J., Morris, R. G., and Wyllie, A. H. (1990) Am. J. Pathol. 136, 593–608
4. Temei, L. D., Shapiro, J. P., and Cope, F. O. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 855–857
5. Walker, P. R., and Sikorska, M. (1994) Biochem. Cell Biol. 72, 615–623
6. Lagarkova, M. A., Iarovaia, O. V., and Raxin, S. V. (1995) J. Biol. Chem. 270, 20239–20241
7. Widom, J., and Klug, A. (1985) Cell 43, 207–213
8. Zlatanov, J., Leuba, S. H., Yang, G., Bustamante, C., and van Holde, K. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 5277–5280
9. Hansen, J. C., Ausio, J., Stark, V. H., and van Holde, K. E. (1989) Biochemistry 28, 9129–9136
10. Russo, I., Barboro, P., Alberti, I., Parodi, I., Balbi, C., Allera, C., Lazzarini, G., and Patrone, E. (1995) Biochemistry 34, 301–311
11. Wyllie, A. H., Morris, R. G., Smith, A. L., and Dunlop, D. (1984) J. Pathol. 142, 67–77
12. Cavazza, B., Brizziolara, G., Lazzarini, G., Patrone, E., Piccardo, M., Barboro, P., Parodi, S., Pasini, A., and Balbi, C. (1991) Biochemistry 30, 9060–9072
13. Barboro, P., Pasini, A., Parodi, S., Balbi, C., Cavazza, B., Allera, C., Lazzarini, G., and Patrone, E. (1993) Biophys. J. 65, 1690–1699
14. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Vol. I, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
15. Sopo, J. M., and Thomay, F. (1989) Methods Enzymol. 170, 142–165
16. Loidl, P., and Grohner, P. (1986) Nucleic Acids Res. 14, 3746–3762
17. Nickel, B. E., Roth, S. Y., Cook, R. G., Allis, D., and Davie, J. R. (1987) Biochemistry 26, 4135–4145
18. Zhang, D., and Nelson, D. A. (1986) Biochem. J. 240, 857–862
19. Laemmli, U. K. (1970) Nature 227, 680–685
20. Wyllie, A. H., and Morris, R. G. (1982) Am. J. Pathol. 109, 78–87
21. Reddi, K. K. (1967) Biochemistry 67–77
22. Nickerson, J. A., Kroehmals, J. C., Wan, K. M., and Penman, S. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 177–181
23. Oberhammer, F., Fritsch, G., Schmied, M., Pavelka, M., Printz, D., Purcio, T., Lassmann, H., and Schulte-Hermann, R. (1993) J. Cell Sci. 104, 317–326
24. Sun, X-M., Snowden, R. T., Dinsdale, M. G., and Cohen, G. M. (1994) Biochem. Pharmacol. 47, 187–195
25. Nicolini, C., Treflett, V., Cavazza, B., Cuniberti, C., Patrone, E., Carlo, P., and Brambilla, G. (1983) Science 219, 176–178
26. Balbi, C., Abelmoschi, M. L., Geggioso, L., Parodi, S., Barboro, P., Cavazza, B., and Patrone, E. (1989) Biochemistry 28, 3220–3227
27. Labarbe, R., Flock, S., Maus, C., and Houssier, C. (1996) Biochemistry 35, 3539–3547
28. Allan, J., Harborne, N., Rau, D. C., and Gould, H. (1982) J. Cell Biol. 93, 285–297
29. Zhang, D., and Nelson, D. A. (1988) Biochem. J. 250, 233–240
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