Histone modifications in the yeast *S. cerevisiae*

J.R.Davie, C.A.Saunders, J.M.Walsh and S.C.Weber

Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331, USA

Received 7 January 1981

ABSTRACT

The content of the acetylated histone species associated with the highly transcriptionally active chromatin of yeast was examined. We found yeast chromatin to contain very high levels of the acetylated species for histones H3, H4 and possibly the H2B variants, H2B-1 and H2B-2. Sixty-three percent of the histone H4 species was represented by the di-, tri- and tetra-acetylated forms. These results make yeast chromatin among the most highly acetylated of any chromatins reported thus far. In addition, the results are consistent with the idea that hyperacetylation of histones allows chromatin to be transcribed at an increased rate.

INTRODUCTION

The yeast histones have been isolated and identified (1,2,3). The four core histones of yeast chromatin include histones H3, H4 and two variant forms each of the histones H2A and H2B (3). A protein which was suggested to be histone H1 (4) has been found to correspond to HMGA (5).

The histones of multicellular eukaryotes can be enzymatically acetylated in two distinct ways (6). In the first type, acetylation occurs at the 2-amino group of the amino-terminal serine of histones H1, H2A and H4. This type of acetylation follows soon after synthesis and is essentially irreversible. The second type occurs at the ϵ -amino groups of the lysyl residues at the amino-terminal portion of the core histones. This type of acetylation is a reversible modification (6-8).

It has been suggested that histone acetylation is involved in chromatin assembly. Shortly after their synthesis, histones are sequentially modified by acetylation and then by deacetylation (6,9,10). This process may mediate the correct interaction of the histone with DNA.

Histone acetylation has also been correlated with increased tran-

scriptional activity in various systems (11-13). Several groups have reported evidence to support the idea that increased histone acetylation may alter the structure of a chromatin region in such a fashion as to be favorable for transcription. DNase I has been demonstrated to preferentially digest transcriptionally competent regions of chromatin (14,15) and, interestingly, chromatin that contains hyperacetylated histones is more readily attacked by DNase I (16-19) than that which does not contain hyperacetylated histones. Also, a chromatin fraction containing high levels of acetylated histone species can be obtained by using procedures that selectively solubilize the transcriptionally competent regions of chromatin (20-23). Recently, Dobson and Ingram (24) have reported that the rate of elongation and, possibly, the rate of initiation for transcription is greater for chromatin containing hyperacetylated histones than that of chromatin containing low levels of acetylated histone species.

Multiple acetylations of the N-terminal portion of the molecule may reduce nucleosome-nucleosome interactions which would allow a region of chromatin to become extended (16,25-27). Recent studies have reported that histone acetylation may also modulate the structure of the nucleosome (16, 28). These structural alterations may be a prerequisite for a region of chromatin to be transcribed at an increased rate (27).

Yeast provides an excellent system for studying the structure of transcriptionally active chromatin as the majority of the chromatin is in a transcriptionally competent conformation (29). This makes it especially interesting to examine the level of histone acetylation in this organism. We have examined the content of the acetylated histone species in various yeast strains and compared the modified histone content of yeast to that associated with both chromatins of low transcriptional activity (calf thymus) and chromatins that are inactive (chick erythrocyte).

MATERIALS AND METHODS

Strains and Growth Conditions

The strains employed in these studies were Y-55, a wild type diploid; A364A haploid; D5, a diploid of A364A; 20B-12 α , a haploid carrying the pep₄₋₃ mutation which reduced the levels of proteases A, B and C ~95% (30).

Strains were grown on YEPD (yeast extract 1%, Bacto peptone 2%, dextrose 2%, supplemented with 40 μ g/ml each of adenine and uracil) at 30°C on a New Brunswick rotary platform shaker to the appropriate growth stage.

Isolation of Nuclei

I. Yeast: Cells in the vegetative phase were harvested by centrifugation at $2,500 \times g$ for 2 min in a Sorvall GS3 rotor and washed once with distilled water containing the indicated amount of sodium butyrate. From this point on, sodium butyrate was added in the indicated concentrations. Cells were repelleted and resuspended in S buffer (1.1 M Sorbitol, 20 mM KH_2PO_4 (pH 6.5), 0.5 mM CaCl_2 , 0.5% β -mercaptoethanol) 4 ml/gm wet weight and Zymolyase 5000 (Kirin Breweries) 150 mg/ml and spheroplasted at 35°C . The spheroplasts were then harvested by centrifugation at $4,000 \times g$ for 5 min in a Sorvall SS-34 rotor. All further manipulations were at 4°C using buffers treated with 1 mM PMSF. The pelleted spheroplasts were homogenized in 0.1 ml/gm of 18% Ficoll 4000 (Pharmacia) using a loose fitting Teflon homogenizer. This step lyses the cytoplasmic membrane and release of the nuclei is monitored microscopically. The mixture was then diluted with an equal volume of 1.0 M Sorbitol, 0.5 mM CaCl_2 and loaded onto a preformed Percoll gradient (the gradients were formed by mixing the appropriate volumes of 1.0 M Sorbitol, 0.5 mM CaCl_2 , pH 6.5, and 100% Percoll 1.0 M Sorbitol (prepared using Percoll as the solvent), 0.5 mM CaCl_2 , pH 6.5). Haploid nuclei were loaded onto 32.5% gradients, diploids 35% gradients. The gradients were preformed by centrifugation at $27,000 \times g$ for 50 min in a Sorvall SS-34 rotor. The nuclei were recovered from the middle of the gradient after centrifugation for 15 min at $9,500 \times g$ in a Sorvall HB-4 rotor. The nuclei were then diluted with 2 volumes of 1.0 M Sorbitol, 0.5 mM CaCl_2 , pH 6.5, and pelleted by centrifugation at $4,000 \times g$ for 5 min in a Sorvall SS-34 rotor. A detailed characterization of the nuclear isolation procedure will be published elsewhere (31).

II. Calf thymus and chicken erythrocytes: Calf thymus tissue or packed erythrocytes from chicken were prepared essentially as described by Weischet *et al.* (32) and by Shaw *et al.* (33), respectively, except the Wray and Stubblefield buffer (34) was modified to include the following: 1 M hexylene glycol, 0.1 mM PIPES (pH 7.0), 1 mM CaCl_2 , 2 mM MgCl_2 , 15 mM β -mercaptoethanol and 30 mM sodium butyrate. In addition, the cells were homogenized in a Potter-Elvehjem homogenizer with a motor-driven Teflon pestle. All isolation buffers contained 30 mM sodium butyrate.

Preparation of histones

Histones were extracted from calf thymus or chicken erythrocyte by resuspending the nuclei with H_2O and adding 4 N H_2SO_4 to a final concentration of 0.4 N. After incubation on ice for 30 min, the insoluble material

was removed by centrifugation. The supernatant fraction was dialyzed against 0.1 N acetic acid and lyophilized.

To prepare histones from yeast, the nuclei were resuspended in Buffer A (10 mM Tris-HCl, pH 8.0, 0.5% NP-40, 75 mM NaCl and, in some cases, 30 or 100 mM sodium butyrate was added). After 20 to 30 min incubation on ice, the nuclei were collected by centrifugation. The above step was repeated two more times. Nuclei were then resuspended in Buffer B (10 mM Tris-HCl, pH 8.0, 0.4 M NaCl and 30 or 100 mM sodium butyrate was added when necessary). After 30 min incubation on ice, the nuclei were collected by centrifugation and resuspended in Buffer B. After centrifugation, the pellet was acid extracted four times as described above.

Polyacrylamide gel electrophoresis

All electrophoresis was done by using a microslab apparatus (35) (Idea Scientific, Corvallis, Oregon). Fifteen percent polyacrylamide-sodium dodecyl sulfate slab gels were made as described by Laemmli (36). Acid-urea slabs were made according to Panyim and Chalkley (37) (2.5 M urea was used) with modifications as described by Spiker (38). Acid-urea Triton X-100 gels were made according to Zweidler (39) (6 mM Triton X-100, 6.7 M urea) with modification as described by Spiker (38). Two-dimensional gels with acid-urea in the first dimension and polyacrylamide-SDS in the second dimension were run essentially as described by Davie and Candido (23), but using the polyacrylamide-SDS slab gel system of Laemmli (36). Two-dimensional gels with acid-urea in the first dimension and acid-urea Triton X-100 in the second dimension were prepared as follows: gel slices from the first dimension were briefly equilibrated (10 min) in 6 mM Triton X-100 and then applied horizontally to the top of the acid-urea Triton X-100 slab gel. The slice was sealed with melted 1% agarose in sample buffer (38). Gels were stained with 0.25% Coomassie blue G-250 in 45% methanol and 9% acetic acid. Gels were destained briefly (30 to 60 min) by diffusion in methanol/acetic acid/water (2:1:5 vol/vol) and then destained further in 7.5% acetic acid and 5% methanol.

Quantification of modified histone species

The spot of interest was cut out from the two-dimensional gel and placed with 70 μ l of 25% pyridine as described by Fenner *et al.* (40). After 12 hr, the absorbance at 595 nm of the resulting solution was determined by using a 1.5 mm silanized microcuvette and a microcuvette holder designed for the Cary 14.

RESULTS

The content of the modified histone forms from different strains of yeast, *Saccharomyces cerevisiae*, has been examined. The strains utilized in this study were Y55, a wild-type diploid from H. O. Halvorson; 20B-12 α , a haploid carrying the pep_{4-3} mutation which reduces the level of proteases A, B and C roughly 95% (30); A364A, a haploid; and the diploid of the same strain, D5.

Nuclei were isolated in sodium butyrate containing buffers from the above yeast strains, chicken erythrocytes, calf thymus and HeLa cells. Sodium butyrate has been reported to inhibit the histone deacetylating enzymes of multicellular eukaryotes (41-44). Preliminary studies suggest sodium butyrate will also inhibit the yeast histone deacetylases. If cells are grown in the presence of sodium butyrate, the acetylated histone species will accumulate (45). To prepare hyperacetylated histones, HeLa cells were grown for 24 hr in the presence of 5 mM sodium butyrate.

Acid soluble nuclear proteins from the yeast strain Y55, calf thymus, chicken erythrocyte and HeLa nuclei were resolved by two-dimensional electrophoresis, acid-urea gel electrophoresis in the first dimension followed by SDS-polyacrylamide gel electrophoresis in the second. Figures 1a, b and c demonstrate the position of the core histones (H3, H2A, H2B, H4) from calf thymus, chicken erythrocyte and HeLa as well as their associated modified forms. The assignment of the yeast core histones (Fig. 1d and e) was made possible from their known electrophoretic migration positions on acid-urea gels and on SDS-polyacrylamide gels (2,5).

The yeast histone H4 was composed of five species which presumably corresponds to the unacetylated (A_0), mono- (A_1), di- (A_2), tri- (A_3) and tetraacetylated (A_4) species. The histone H4 heterogeneity was attributed to acetylation because 1) the yeast histone H4 can be separated into five species and resolved in a similar position on the two-dimensional gels as the known HeLa H4 acetylated species (Fig. 1c); and 2) if sodium butyrate was not present during the nuclear isolation, the distribution of the histone H4 species would shift to the right (compare Fig. 1d and e) which was probably due to the accumulation of the histone H4 A_0 and A_1 species at the expense of the A_2 , A_3 and A_4 species.

The yeast histone H3 was likely acetylated as well because "tailing" was observed when the nuclei were isolated in the presence of sodium butyrate, but not in its absence (compare Fig. 1d and e).

The histone H3 and H4 heterogeneity cannot be due to variant forms that

Figure 1. Two-dimensional electrophoretic patterns of histones extracted with H_2SO_4 from nuclei of calf thymus, chicken erythrocytes, HeLa and yeast. Acid soluble nuclear proteins were resolved by acid-urea gel electrophoresis in the first dimension and by 15% polyacrylamide-SDS gel electrophoresis in the second dimension. A, chicken erythrocyte; B, calf thymus; C, HeLa (cells grown in the presence of 5 mM sodium butyrate for 21 hours); D and E, yeast strain Y55 (nuclei were isolated in the presence or absence of 100 mM sodium butyrate, respectively). X, Y and Z are unidentified protein(s).

differ by one charge, as Brandt *et al.* (3) have recently reported that there was only one variant form for histones H3 and H4.

The heavy bands that were present in Fig. 1(e) but not in Fig. 1(d) could be removed if the nuclei were washed more extensively with Buffer A (see Materials and Methods).

These studies demonstrate that to observe the normal levels of acetylated histone species, it was essential to have sodium butyrate present in the isolation medium. During the course of these studies, we observed that the sodium butyrate concentration required to fully protect the existing acetylated species from deacetylation was dependent on the spheroplasting time and the method of nuclear isolation (e.g., long spheroplasting times

required higher concentrations of sodium butyrate.

The content of the acetylated histone H4 species isolated from the nuclei of calf thymus, chicken erythrocyte, yeast (Y55, butyrate present) and yeast (Y55, butyrate absent) was quantified (Table I). The histone H4 A₂ species of yeast were most abundant if sodium butyrate was present during the nuclear isolation, but the histone H4 A₁ species were predominant if sodium butyrate was not present. A similar distribution of acetylated histone H4 species was observed for the different strains examined. In all cases when butyrate was present in the isolation buffers, the diacetyl H4 species predominated [see Fig. 1 (Y55, Table I, and A364A and D5, not shown)]. Similar levels of acetylated histone H4 species were observed if the histones were extracted from either nucleosomes (20B-12α strain was used with 30 mM sodium butyrate present during the isolation) or from chromatin rapidly prepared by breaking cells with glass beads (Y55, 100 mM sodium butyrate present during the isolation).

The extent of yeast histone H4 acetylation was much higher than that of chicken erythrocyte or calf thymus. Chicken erythrocyte contained the lowest proportions of the acetylated species. The yeast strains contained a 4 to 5 fold increase in the content of A₂, A₃ and A₄ histone H4 species compared to the distribution of these acetylated species from calf thymus or chicken erythrocyte. Even with butyrate absent from the isolation medium,

Table I. The percentage of acetylated histone H4 species in preparations of whole chromatin.

Cells	A ₀	A ₁	A ₂	A ₃	A ₄
%					
Calf thymus	44 ± 4	43 ± 3	13 ± 6	-	-
Chicken erythrocytes	59 ± 1	30 ± 4	11 ± 3	-	-
<u>Yeast</u>					
Y55 (control)	17 ± 3	52 ± 2	31 ± 3	-	-
Y55 (+100 mM sodium butyrate)	10 ± 2	26 ± 3	32 ± 3	21 ± 3	11 ± 4
20B-12α(+30 mM sodium butyrate)	14 ± 2	27 ± 1	31 ± 3	17 ± 4	11 ± 2

yeast had a three-fold increase in the content of the above species over those associated with calf thymus or chicken erythrocyte.

The histones from logarithmically growing yeast (20B-12 α) cells were examined further by resolving the nuclear proteins by a two-dimensional gel electrophoresis system which consisted of an acid-urea gel in the first dimension followed by an acid-urea Triton X-100 gel in the second. We chose to use the conditions of Zweidler (39) for preparation of the acid-urea Triton X-100 gels (6 mM Triton X-100, 6.7 M urea). Figure 2 presents the electrophoretic pattern of the nuclear proteins resolved by this system. The assignment of the histones was made by dissecting out the bands and identifying the histone on SDS-polyacrylamide gels. In

Figure 2. Two-dimensional electrophoretic patterns of acid soluble nuclear proteins from yeast strain 20B-12 α . Acid soluble nuclear proteins were resolved by acid-urea gel electrophoresis in the first dimension and by acid-urea Triton X-100 gel electrophoresis in the second dimension. Nuclei isolated in the presence of 30 mM sodium butyrate were obtained from vegetative phase cells. X, Y and Z are unidentified protein(s).

addition, the positions of an unidentified protein, X, and of a group of unidentified proteins, Y and Z, shown in the electrophoretic pattern (Fig. 1e) were determined for the electrophoretic pattern (Fig. 2).

In agreement with the results of Brandt *et al.* (3), we have identified two variant forms of yeast histone H2B. Both variant forms of H2B contained a number of modified species (7 species for H2B-2 and 5 species for H2B-1) similar to those observed for histone H4.

The gel systems that were used contained ammonium persulfate in the stacking gel. This was found to oxidize the H2B methionine (39). If the stacking gel was omitted from both gels and the sample reduced prior to electrophoresis (40% thiglycolic acid, 12h, 50°), the H2B-2 migrated slower than H2B-1 on the two-dimensional gels. In addition, there were no changes in their content of modified forms or the relative amounts of each variant.

The modified species of histones H2B-1 and H2B-2 may be due to acetylation, phosphorylation or a combination of both. However, extensive digestion of the histones with acid phosphatase did not alter the observed content of either of the major species of the histone H2B variants or the species of histone H4. Thus, the results suggest the major modification of the histone H2B variants may be acetylation.

DISCUSSION

We have examined the content of modified histone species associated with different yeast strains, 20B-12 α , Y55, A364a and D5. The yeast strains contained similar levels of modified species each of the histones H3, H4, H2B-1 and H2B-2. For histones H3 and H4 the modification was identified as acetylation (see Results).

The content of modified species for each of the histone H2B variants, H2B-1 and H2B-2 were similar. The major species were probably not phosphorylated as digestion of the histones with acid phosphatase did not alter their patterns on two-dimensional gels. The modification may be acetylation, but we hesitate to make this assignment because the distribution of the modified forms did not alter when sodium butyrate was present or absent during the nuclear isolation. The exact nature of this modification(s) will have to await results from labeling studies.

During the course of this work, we have found that the presence of sodium butyrate in the isolation buffers was essential in order to isolate the highly acetylated histone species. To isolate the highly acetylated

histone species, one must select the correct concentration of sodium butyrate by taking into consideration the following: 1) the yeast strain to be used, 2) the time of spheroplasting required, and 3) the nuclei isolation procedure to be used.

Several groups have reported that the histones of multicellular eukaryotes are acetylated and deacetylated at different rates (7,8,46,47). Moore *et al.* (7) have reported two types of acetylation: one in which 50% of the acetate was removed with half-life of 3 min and the rest with a much longer half-life of 30 to 40 min. Recently, Nelson *et al.* (46) have demonstrated that the transcriptionally competent chromatin regions contain histones that are more rapidly and extensively acetylated than that of histones associated with the bulk of chromatin. Their results would suggest that the very transcriptionally active chromatin of yeast might contain a greater proportion of histones that are rapidly hyperacetylated and deacetylated. It will be interesting to examine the turnover rate(s) of the yeast histone acetyl groups. However, we were concerned that the presence of sodium butyrate during the spheroplasting step during which the cells were still metabolically active would lead to a rapid accumulation of acetylated histone species while the deacetylase(s) were inhibited. This would result in an increased content of the highly acetylated histone species and would not represent the *in vivo* content of these species. We feel that this was not occurring because: (1) chromatin prepared by rapid disruption of vegetative phase cells by glass beads in the presence of 100 mM sodium butyrate contained high levels of acetylated histone species, and (2) we obtained histones that were extracted from vegetative phase nuclei of the yeast strain, 20B-12 α , isolated in the absence of sodium butyrate that contained high levels of acetylated histone species similar to those extracted from nuclei isolated in the presence of sodium butyrate (30 or 100 mM).

Our results are consistent with the idea that hyperacetylation of histones allows a region of chromatin to be transcribed at an increased rate. We have compared chromatins that vary dramatically in their transcriptional activities. The very transcriptionally active chromatin of yeast contains 4 to 5 times the content of the A₂, A₃ and A₄ histone H4 as that of both the slightly transcriptionally active chromatin of calf thymus and the transcriptionally inactive chromatin of chicken erythrocyte. Furthermore, yeast contains high levels of the acetylated species for histone H3 and, possibly, for the histones H2B-1 and H2B-2 which would make

yeast chromatin among the most highly acetylated of any chromatins reported thus far.

ACKNOWLEDGMENTS

The authors wish to acknowledge Dr. K. E. Van Holde for providing laboratory space and support from a USPHS grant (GM 22106) as well as helpful suggestions. JRD was supported by a post-doctoral fellowship from the MRC (Canada). CAS was a USPHS fellow (GM-96922). JMW was supported by a predoctoral training grant from NIH (GM-07774). SCW was supported by a grant from NIH (CA 10272, to I. Isenberg). We are also indebted to F. Ziemer for the gift of HeLa histones and to I. Isenberg for a critical reading of this manuscript. We are very grateful to Dr. Beth Jones for making the yeast strain 20B-12 α available to us and for helpful discussions.

REFERENCES

1. Nelson, D.A., Beltz, W.R. and Rill, R.L. (1977) Proc. Natl. Acad. Sci. USA 74, 1343-1347.
2. Mardian, J.K.W. and Isenberg, I. (1978) Biochem. 17, 3825-3833.
3. Brandt, W.F., Patterson, K. and von Holt, C. (1980) Eur. J. Biochem. 110, 67-76.
4. Sommer, A. (1978) Mol. Gen. Genet. 161, 323-331.
5. Weber, S. and Isenberg, I. (1980) Biochem. 19, 2236-2240.
6. Dixon, G.H., Candido, E.P.M., Honda, B.M., Louie, A.J., MacLeod, A.R. and Sung, M.T. (1975) CIBA Foundation Symposium 28: The Structure and Function of Chromatin, Ed. Wolstenholme, D. (Churchill and Livingston, Edinburgh and London), pp. 229-258.
7. Moore, M., Jackson, V., Sealy, L. and Chalkley, R. (1979) Biochim. Biophys. Acta 561, 248-260.
8. Jackson, V., Shires, A., Chalkley, R. and Granner, D.K. (1975) J. Biol. Chem. 250, 4856-4863.
9. Louie, A.J. and Dixon, G.H. (1972) Proc. Natl. Acad. Sci. USA 69, 1975-1979.
10. Ruiz-Carrillo, A., Wangh, L.J. and Allfrey, V.G. (1975) Science 190, 117-128.
11. Allfrey, V.G. (1977) in: Chromatin and Chromosome Structure, Eds. H.J. Li and R.A. Eckhardt, Academic Press, New York, pp. 167-191.
12. Gorovsky, M.A., Glover, C., Johmann, C.A., Keever, J.B., Mathis, D.J., and Sunvelson, M. (1977) Cold Spring Harbor Symp. Quant. Biol. 42, 493-503.
13. Chestier, A. and Yaniv, M. (1979) Proc. Natl. Acad. Sci. USA 76, 46-50.
14. Garel, A. and Axel, R. (1976) Proc. Natl. Acad. Sci. USA 73, 3966-3970.
15. Weintraub, H. and Groudine, M. (1976) Science 193, 848-856.
16. Simpson, R.T. (1978) Cell 13, 691-699.
17. Mathis, D.J., Oudet, P., Wasylyk, B. and Chambon, P. (1978) Nucleic Acids Res. 5, 3523-3547.
18. Vidali, G., Boffa, L.C., Bradbury, E.M. and Allfrey, V.G. (1978) Proc. Natl. Acad. Sci. USA 75, 2239-2243.
19. Davie, J.R. and Candido, E.P.M. (1980) FEBS Lett. 110, 164-168.

20. Nelson, D.A., Perry, W.M. and Chalkley, R. (1978) *Biochem. Biophys. Res. Commun.* 82, 356-363.
21. Sealy, L. and Chalkley, R. (1978) *Nucleic Acids Res.* 5, 1863-1876.
22. Keuhl, L., Lyness, T., Dixon, G.H. and Levy-Wilson, B. (1980) *J. Biol. Chem.* 255, 1090-1095.
23. Davie, J.R. and Candido, E.P.M. (1978) *Proc. Natl. Acad. Sci. USA* 75, 3574-3577.
24. Dobson, M.E. and Ingram, V.M. (1980) *Nucleic Acids Res.* 8, 4201-4218.
25. Whitlock, Jr., J.P. and Stein, A. (1978) *J. Biol. Chem.* 253, 3857-3861.
26. Pospelov, V.A., Svetlikova, H.M. and Vord'ev, V.I. (1979) *FEBS Lett.* 99, 123-128.
27. Chahal, S.S., Matthews, H.R. and Bradbury, E.M. (1980) *Nature* 287, 76-79.
28. Bode, J., Henco, K. and Wingender, E. (1980) *Eur. J. Biochem.* 110, 143-152.
29. Lohr, D. and Hereford, L. (1979) *Proc. Natl. Acad. Sci. USA* 76, 4285-4288.
30. Jones, E. (1977) *Genetics* 85, 23.
31. Ide, G. and Saunders, C. (1980) *Curr. Genet.* (submitted for publication).
32. Weischet, W.O., Allen, J.R., Riedel, G. and Van Holde, K.E. (1979) *Nucleic Acids Res.* 6, 1843-1862.
33. Shaw, B.R., Herman, T.M., Kovacic, R.T., Beaudreau, G.S. and Van Holde, K.E. (1976) *Proc. Natl. Acad. Sci.* 73, 505-509.
34. Wray and Stubblefield (1970) *Expt. Cell Research* 59, 469-478.
35. Matsudaira, P.I. and Burgess, D.R. (1978) *Anal. Biochem.* 87, 386-396.
36. Laemmli, U.K. (1971) *Nature (London)* 227, 1.
37. Panyim, S. and Chalkley, R. (1969) *Arch. Biochem. Biophys.* 130, 337-346.
38. Spiker, S. (1980) *Anal. Biochem.* 108, 263-265.
39. Zweidler, A. (1978) *Methods Cell Biol.* 17, 223-265.
40. Fenner, C., Traut, R.R., Mason, D.T. and Wikman-Coffelt, J. (1975) *Anal. Biochem.* 63, 595-602.
41. Boffa, L.C., Vidali, G., Mann, R.S. and Allfrey, V.G. (1978) *J. Biol. Chem.* 253, 3364-3366.
42. Sealy, L. and Chalkley, R. (1978) *Cell* 14, 115-121.
43. Cousens, L.S., Gallwitz, D. and Alberts, B. (1979) *J. Biol. Chem.* 254, 1716-1723.
44. Candido, E.P.M., Reeves, R. and Davie, J.R. (1978) *Cell* 14, 105-113.
45. Riggs, M.G., Whittaker, R.G., Neumann, J.R. and Ingram, V.M. (1977) *Nature* 268, 462-464.
46. Nelson, D., Covault, J. and Chalkley, R. (1980) *Nucleic Acids Res.* 8, 1745-1763.
47. Covault, J. and Chalkley, R. (1980) *J. Biol. Chem.* 255, 9110-9116.