Sequence Analysis of Acetylation and Methylation in Two Histone H3 Variants of Alfalfa*

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Analysis of acetylation in the two histone H3 variants of alfalfa by acid/urea/Triton-polyacrylamide gel electrophoresis has established that the major variant H3.2 has a 2-fold higher level of acetylation than the major variant H3.1. Purification and sequence analysis of both variants showed sequence identity across the complete amino-terminal domain, which contains the 6 modified lysines 4, 8, 14, 18, 23, and 27. The two proteins have different distributions for acetylation: mono-, di-, and tri-methylation. The higher level of acetylation of H3.2 was confirmed in a wider pattern across all 6 lysines. Lysine modification levels varied for all sites in both proteins between 6 and 65%, with combinations of one to four types of modification co-existing at each residue. Additional sequence analysis of the H3.1 and H3.2 proteins and of tryptic core peptides established that the two histones differ only in residues 31, 41, 87, and 90. This indicates that major histone H3.1 is the product of the major alfalfa histone H3 gene and makes it likely that H3.2 is the product of the minor H3 gene, known from a partial cDNA clone. The variant-specific differences in lysine modifications in protein domains with identical primary structures suggest that the pattern and level of lysine modifications may be directed by the distinct chromatin environments of the two histone H3 variants.

EXPERIMENTAL PROCEDURES

Preparation of Alfalfa Histones—Friable calli of alfalfa, cultivar strain R4 of Medicago sativa, were grown for 4 weeks at 25 °C, 85% relative humidity, and constant light. Aliquots of 200 g of fresh callus were dispersed and homogenized in a precooled 1-liter Waring Blender with a rotor-stator knife assembly 3 times for 1 min at high speed of 22,000 × g, and acidified by addition of concentrated HCl to 0.25 N. The solution was stirred on ice for 15 min, clarified by centrifugation for 30 min at 22,000 × g, diluted with 0.1 M potassium phosphate at pH 6.8, neutralized with KOH to pH 6.8, clarified by centrifugation for 20 min at 6,200 × g, and adjusted to 0.5 mM 2-mercaptoethanol. Bio-Rex-70 resin (Bio-Rad), equilibrated with buffer G5, was added to a ratio of 1 ml of resin for each 30 g of homogenized callus. The suspension was stirred at room temperature overnight. The resin was allowed to settle, washed with buffer B (0.1% trifluoroacetic acid in acetonitrile) containing 1.5 mM 2-mercaptoethanol, packed in a column, and washed with 8 column volumes of buffer G5 with 2-mercaptoethanol. The crude histone preparation was eluted by 10 column volumes of buffer G40 containing 1.5 mM 2-mercaptoethanol, dialyzed in Spectra/Por 3 membranes (3500-Da cut-off) against three changes of 100 volumes of 5% (v/v) acetic acid sufficient requirement to allow RNA polymerases to further unfold the chromatin for gene transcription (for reviews, see Matthews and Waterborg, 1985; Gross and Garrard, 1987, 1988; Pederson and Simpson, 1988; Vidali et al., 1988). This model assumes that differences in histone sequence, observed as histone variants, play no role. However, this model cannot explain observed preferential localization of some histone variants in active chromatin (Allis et al., 1986; Donahue et al., 1986; Jasinskiene et al., 1985, 1988) nor their exclusion from it (Jalleck and Gurley, 1982). Moreover, it predicts that all histone variants of a certain histone type will have, on average, identical levels of histone acetylation, whereas the levels of histone H3 acetylation in the two H3 variants of alfalfa (Waterborg et al., 1987, 1989) and tobacco1 are very different.

This study was initiated to evaluate whether the primary structure of the alfalfa histone H3 variants differ sufficiently in the domain containing the acetylation sites to explain the observed differences in acetylation. Sequence analysis of substantial parts of purified H3.1 and H3.2 proteins revealed variant-specific patterns and levels of lysine acetylation and methylation in a protein domain that does not contain the variant-specific differences in primary structure. Histone H3 variant modifications may be determined by the chromatin environment.

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1 J. H. Waterborg, unpublished results.
with 1.5 mM 2-mercaptoethanol, and lyophilized.

Preparative amounts of histones from up to 1500 g of callus were fractionated on a 2.5 x 100-cm column of Bio-Gel P60 (Bio-Rad) in 20 mM HCl, 50 mM NaCl, 0.02% sodium azide (Von Holt and Brandt, 1977) as described previously (Waterborg et al., 1987). Fractions containing histone H3 were identified by acid/urea/Triton (AUT) and HPLC chromatography of Zorbax Protein Plus as described under "Experimental Procedures" and shown as the lower and upper registrations of the absorbance at 214 nm, respectively. Histone variants H3.1 and H3.2 eluted separately during a gradient elution between 42 and 52% buffer B (acetonitrile in water/0.1% trifluoroacetic acid, dotted line). Samples across the double elution peaks of the upper chromatographic registration were dried and analyzed by AUT (lanes 1-8) and SDS (lanes 9-12) gel electrophoreses, as indicated. All samples containing high concentrations of histone H3 show to a small and variable degree apparent oxidation to dimers (arrowhead). Calf thymus histones were used as markers in AUT (lane m with histones H2A, H1, H2B, and H4 indicated from top to bottom) and SDS gel electrophoreses (lane M with histones H1, H3, H2B, H2A, and H4 indicated from top to bottom). Samples corresponding to AUT, lanes 3 and 4, and 6 and 7, or SDS, lanes 10 and 11, were routinely used for sequence analysis. The basis for the peak heterogeneity observed for both histone H3 variant peaks has not yet been identified and is not detectable by AUT or SDS gel analyses.

![Image of reversed-phase HPLC of alfalfa histone H3 variants](image.png)

**Fig. 1.** Reversed-phase HPLC of alfalfa histone H3 variants. Histone H3 proteins, extracted from callus cultures of alfalfa, were prefractionated by Bio-Gel P60 chromatography, and extracts corresponding to 28 and 280 g of callus were chromatographed on Zorbax Protein Plus as described under "Experimental Procedures" and shown as the lower and upper registrations of the absorbance at 214 nm, respectively. Histone variants H3.1 and H3.2 eluted separately during a gradient elution between 42 and 52% buffer B (acetonitrile in water/0.1% trifluoroacetic acid, dotted line). Samples across the double elution peaks of the upper chromatographic registration were dried and analyzed by AUT (lanes 1-8) and SDS (lanes 9-12) gel electrophoreses, as indicated. All samples containing high concentrations of histone H3 show to a small and variable degree apparent oxidation to dimers (arrowhead). Calf thymus histones were used as markers in AUT (lane m with histones H2A, H1, H2B, and H4 indicated from top to bottom) and SDS gel electrophoreses (lane M with histones H1, H3, H2B, H2A, and H4 indicated from top to bottom). Samples corresponding to AUT, lanes 3 and 4, and 6 and 7, or SDS, lanes 10 and 11, were routinely used for sequence analysis. The basis for the peak heterogeneity observed for both histone H3 variant peaks has not yet been identified and is not detectable by AUT or SDS gel analyses.

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**Analysis of Histone H3 Variants**—SDS and AUT gel electrophoreses, fluorography, and gel quantitation were performed as described previously (Waterborg et al., 1987, 1989). Histone H3 proteins and peptides were hydrolyzed for 24 h at 110 °C in 6 n constant-boiling HCl with 0.1% phenol, and the amino acid composition was determined by HPLC (Bio-Rad) and postcolumn o-phthalaldehyde derivatization, which preceded detection of proline. Prehydrolysis derivatization of cysteine to a stable derivative was not performed.

**Automated Sequence Analysis**—The standard protein sequencing protocol for Edman degradation and phenylthiohydantoin-derivative identification from an Applied Biosystems 477A/120 Pulsed-liquid Phase Sequencer was used with repetitive yields of 92% or higher. Extended proline digestion cycles were used at positions 16, 30, 38, and 43 during the sequencing of intact histone H3 proteins. Automated cyanogen bromide digestion of the small overlap between the two peaks after preparative chromatography. Analytical retention times were 45 min for histone H3.1 and 49 min for H3.2 (Fig. 1). The pooled histones were judged to be homogeneous by SDS and AUT gel electrophoreses. A typical recovery of homogeneous H3 variants was 0.6 mg of H3.1 and 0.5 mg of H3.2 protein/g of callus. For the analysis on the total number of detectable acetylation sites by fluorography (Fig. 2), the small overlap between the two histone H3 variants was not discarded to avoid possible bias, but care was taken to avoid inclusion of H3.1 protein into the H3.2 fraction because H3.1 protein was not discarded to avoid possible bias, but care was taken to avoid inclusion of H3.1 protein into the H3.2 fraction (Fig. 2).

**Isolation of the Tryptic Core of Histone H3**—HPLC-separated histone H3 variants were dissolved in 50 mM NH4HCO3, pH 8.2, to 1 mg/ml and digested with 1/40 weight of L-1-lysylamido-2-phenylthiocarbamyl ketone-treated trypsin (Worthington) twice for 2 h at 37 °C. The digest was lyophilized and fractionated on a 4.6-mm x 25-cm Zorbax Protein Plus reversed-phase HPLC column in 0.1% trifluoroacetic acid in water which was developed at 0.5 ml/min with a 120-min gradient to 70% acetonitrile in water with 0.1% trifluoroacetic acid. The tryptic core peptide of both histone H3 variants (residues 84-116 (Brandt and Von Holt, 1982; Vanfleteren et al., 1987)) eluted at 90 min and was identified by UV absorbance of tyrosine at 275 nm and by amino acid analysis.

**Analysis of Histone H3 Variants**—SDS and AUT gel electrophoreses, fluorography, and gel quantitation were performed as described previously (Waterborg et al., 1987, 1989). Histone H3 proteins and peptides were hydrolyzed for 24 h at 110 °C in 6 n constant-boiling HCl with 0.1% phenol, and the amino acid composition was determined by HPLC (Bio-Rad) and postcolumn o-phthalaldehyde derivatization, which preceded detection of proline. Prehydrolysis derivatization of cysteine to a stable derivative was not performed.

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**Isolation of the Tryptic Core of Histone H3**—HPLC-separated histone H3 variants were dissolved in 50 mM NH4HCO3, pH 8.2, to 1 mg/ml and digested with 1/40 weight of L-1-lysylamido-2-phenylthiocarbamyl ketone-treated trypsin (Worthington) twice for 2 h at 37 °C. The digest was lyophilized and fractionated on a 4.6-mm x 25-cm Zorbax Protein Plus reversed-phase HPLC column in 0.1% trifluoroacetic acid in water which was developed at 0.5 ml/min with a 120-min gradient to 70% acetonitrile in water with 0.1% trifluoroacetic acid. The tryptic core peptide of both histone H3 variants (residues 84-116 (Brandt and Von Holt, 1982; Vanfleteren et al., 1987)) eluted at 90 min and was identified by UV absorbance of tyrosine at 275 nm and by amino acid analysis.

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RESULTS

The two histone H3 variant proteins of alfalfa (H3.1 and H3.2) can be separated by water/acetonitrile gradients with 0.1% trifluoroacetic acid ion pairing on a Zorbax Protein Plus reversed-phase HPLC column. The two histone H3 proteins elute later than any other histone and are separated from each other by approximately 0.7% acetonitrile. With analytical sample amounts, base-line separation is possible, and at the semipreparative sample amounts of crude histone H3 used in this study, routinely 75% of both variant proteins could be purified to homogeneity by excluding the small overlap between the peaks (Fig. 1).

As a source of histone H3, total cells were used (Waterborg et al., 1987) rather than isolated nuclei (Waterborg et al., 1989). Artificial heterogeneity arises during the preparation of alfalfa nuclei, probably due to some form of cysteine modification in the H3 proteins (Waterborg, 1990). The extraction of histones from total cells rather than from nuclei increased histone recoveries approximately 2-fold but decreased the histone content of the crude extract to below 50%. The maximal capacity of a Zorbax Protein Plus column (4.6 mm × 25 cm) for separation of the histone H3 variants was reached when 5 mg of total protein was applied. Fractionation of the total crude histone preparation by Bio-Gel P60 chromatography (Waterborg et al., 1987) to obtain a histone H3-enriched preparation allowed separation and purification of 10-fold more histone H3 per reversed-phase HPLC run (see "Experimental Procedures"). Moreover, a small contamination of minor H2A variant protein and trace amounts of non-histone proteins were removed from the histone H3 preparations. A single band of identical molecular weight was visible for each preparation in SDS gels (lanes 10 and 11 in Fig. 1). The purity of each histone variant preparation was further proven by amino acid analysis (Table I) and by automated sequence analysis, which indicated a contamination level of less than 1% of non-histone H3 protein.

Analysis of the two purified histone variant proteins by AUT gel electrophoresis showed a series of multiple bands for each protein (lanes 3-4 and 6-7 in Fig. 1) in addition to a variable amount of presumably oxidized histone H3 dimer. To identify acetylation as the cause for this pattern of distinctly charged histone H3 protein forms and to establish the total number of detectable modification sites, alfalfa callus cultures were labeled in vivo with tritiated acetate, histone H3 variant proteins were fractionated by reversed-phase chromatography of the crude histone preparation, and the fractions were analyzed by AUT gel electrophoresis (Fig. 2). Histone H3.1 clearly showed three modified protein bands with increasing discrete reductions in gel mobility relative to the major protein form (Protein 1 in Fig. 2). These modified bands appear to be caused by acetylation, as judged by the acetate label incorporation pattern (Label 1 in Fig. 2), but extensive fluorographic analysis revealed the existence of six possible sites (Fig. 2A). Histone H3.2 had a 2-fold higher level

Table I

| Amino acid | H3.1 | H3.2 | Predicted from gene sequences* |
|------------|------|------|------------------------------|
|             | mol%|      | amino acid no. | mol%               |
| Aax        | 4.4 | 4.5  | 5               | 3.7               |
| Thr        | 7.3 | 8.0  | 10              | 7.4               |
| Ser        | 4.4 | 3.4  | 6               | 4.4               |
| Glx        | 12.4| 12.4 | 15              | 11.1              |
| Pro        | ND* | ND   | 6               | 4.4               |
| Gly        | 6.1 | 6.1  | 7               | 5.2               |
| Ala        | 13.8| 12.9 | 19              | 14.1              |
| Val        | 4.9 | 5.0  | 6               | 4.4               |
| Cys        | ND  | ND   | 1               | 0.7               |
| Met        | 0.3 | 0.3  | 1               | 0.7               |
| Ile        | 5.2 | 5.2  | 7               | 5.2               |
| Leu        | 9.0 | 9.6  | 12              | 8.9               |
| Tyr        | 1.3 | 2.0  | 2               | 1.5               |
| Phe        | 3.4 | 3.0  | 5               | 3.7               |
| Lys        | 10.4| 10.3 | 14              | 10.4              |
| His        | 2.0 | 2.2  | 3               | 1.5               |
| Arg        | 15.1| 14.8 | 17              | 12.6              |
| Total      | 100.0| 100.0| 135             | 126               |

* Prediction is based on Wu et al. (1989), assuming no sequence differences at residues 1-16.
* ND, not determined.
of steady-state acetylation than did H3.1, as determined by
quantitative Gaussian curve-fitting analysis of the Coomassie-
stained pattern (Protein 2, and the continuous scan in Fig.
2B). The higher level of H3.2 acetylation was also supported
by the larger number of detectable acetylation bands (Label
2 in Fig. 2) and their relatively higher abundance in the
fluorography pattern with probably more than six sites of
modification (Fig. 2B).

Automated sequence analysis of both histone H3 variant
proteins for 30 residues revealed identical sequences (Fig. 3).
At lysine residues 4, 9, 14, 18, 23, and 27, additional, uniden-
tified phenylthiohydantoin-derivative peaks were observed
at varying intensities. With the known postsynthetic modifi-
cation of lysine by acetylation and methylation, the elution
pattern of various modified forms of lysine was established
using known standards. Extension of the NOKMAL-1 HPLC
gradient elution program enabled all previously unknown
peaks to be identified as acetylated, mono-, di-, or trimeth-
ylated lysine. With quantitation of these modified residues
relative to unmodified lysine, a complex pattern of acetylation
and methylation was revealed with distinct quantitative and
qualitative differences between histones H3.1 and H3.2 (Table
II). It should be noted that the accuracy of quantitation of
lysine, acetylysine, and monomethyllysine is higher than that
of di- and trimethyllysine, which have lower apparent molar
absorbance values (see “Experimental Procedures”).

To establish the extent of primary structure differences for
the two histone H3 proteins, additional sequence analyses
were performed, guided by the observed differences in the
amino acid analysis between the two variant proteins (Table
I) and by the published cDNA sequence data for the major
histone H3 gene of alfalfa (Win et al., 1998). This study
identified a minor histone H3 gene from a partial cDNA clone
(pH3c.11), predicting a histone H3 protein from amino acid
resides 17–138 which would differ at residues 31, 41, 87, and
90 from the major gene (Fig. 3). Amino acid analysis of histone
H3.1 and H3.2 showed differences only for threonine, serine,
alanine, leucine, tyrosine, phenylalanine, and histidine (Table
1), consistent with the major protein variant H3.1 as the
product of the major histone H3 genes of alfalfa and the minor
protein variant H3.2 as the product of the minor gene(s).

This conclusion was supported by automated sequence analysis
of histone H3.1 for 44 and of H3.2 for 51 residues, which showed
the predicted sequences, including the differences at residues
31 and 41 (Fig. 3), and which supplied the amino-terminal
sequence missing from the partial gene clone. Lysines 36 and
37 in both proteins appeared not to be modified by acetylation
or methylation. Tryptic core peptides were prepared and
sequenced to confirm the predicted sequence differences for
residues 87 and 90 (Fig. 3).

**DISCUSSION**

This study identifies the three major sites of acetylation in
alfalfa histone H3 as lysines 14, 19, and 23. Minor sites of
acetylation were only quantitated in histone variant H3.2 as
lysines 27 and 9, with a trace of modification detected at
lysine 4 (Table II). This shows that the sensitivity for quan-
titation of acetylation by sequencing is similar to that by
quantitative analysis of Coomassie-stained AUT gels (Fig. 2).
Calculation from the sequence data of acetylated lysine residues
per protein molecule for H3.1 and H3.2 yields values of
0.38 and 0.73 acetylysine, respectively. These are similar to
values determined from AUT gel analysis of 0.44–0.56 for
H3.1 and 0.97–1.01 for H3.2. In both analyses, the relative
level of acetylation is estimated to be 2-fold higher for histone
variant H3.2 than for variant H3.1.

Fluorographic detection of the total number of acetylation
sites of alfalfa histone H3 is more sensitive than either
method, but it is limited to a qualitative assessment. At least
seven sites of acetylation were detectable in histone H3.2 (Fig.
2), six of which are presumed to be the 6 lysines observed
to be acetylated in histone H3.2 (Fig. 3). This result may indicate
that, in trace amounts, lysines other than those in the amin-
terminal region of histone H3 may become acetylated. An
alternative explanation would be the generation of modified
forms of histone H3 by modifications other than acetylation.
The involvement of phosphorylation appears excluded by the
failure to label histone H3 in vivo with radioactive inorganic
phosphate (Waterborg et al., 1989).

Histone H3 can be divided into two distinct structural
domains. The amino-terminal domain of residues 1–27 con-
tains all known lysine acetylation and methylation sites and
appears highly accessible in chromatin as judged by its sen-
sitivity towards proteolytic fragmentation, especially near
residue 27 (Rill and Oosterhof, 1982; Marion et al., 1983;
Crane-Robinson and Boehm, 1985; Dimitrov et al., 1986;
Dumnis-Kervabon et al., 1986; Encontre and Parello, 1988;
Ausio et al., 1989). The carboxy terminal part of histone H3
appears to be a structured, globular domain with major hy-
drophobic characteristics. The sequence differences between

**Table II**

| Residue | Histone H3.1 | Histone H3.2 |
|---------|--------------|--------------|
|         | AcL | MML | DML | TML | AcL | MML | DML | TML |
| 4       | 76.1 | 4.3 | 3.8 | 15.7 | 50.3 | 24.4 | 6.4 | 18.9 |
| 9       | 11.2 | 73.5 | 15.2 | 31.5 | 1.6 | 43.4 | 8.7 | 14.8 |
| 14      | 66.2 | 20.2 | 3.6 | 45.3 | 37.0 | 17.6 |
| 18      | 71.2 | 12.8 | 16.9 | 60.6 | 24.7 | 15.7 |
| 23      | 95.1 | 4.9 | 16.9 | 9.0 | 33.1 |
| 27      | 5.2  | 31.0 | 21.5 | 42.3 | 36.2 | 15.6 | 24.1 | 38.1 |
histone H3.1 and H3.2 at residues 31, 41, 87, and 90 all reside in the globular domain. This makes it highly unlikely that these primary structure differences could be the determining factor in the distinct differences in lysine acetylation and methylation within the amino-terminal domain of the histone H3 variants (Table II).

The pattern of acetylation of histone H3.1 covers three contiguous lysines at positions 14, 18, and 23. Acetylation of histone H3.2 is similar, with higher levels of modification of these lysines and low levels in additional, contiguous lysines 27, 9, and 4 (Table II). This pattern suggests that the histone-acetylating enzymes lack a strict sequence specificity and that their action may be directed more by steric determinants. Lysines within the globular domain beyond residue 27, such as lysines 36 and 37, remain completely unmodified. The decrease in acetylation levels from lysine 14 to lysine 27 may be caused by increasing steric interference for acetylation by the neighboring globular histone domain. However, a severe restriction towards acetylation of lysines 9 and 4 is apparent.

It is tempting to speculate that this area of histone H3 is closely associated with some chromatin structural element when histone H3 is part of chromatin. Highly methylated lysine 9 may be important for this interaction, because in histone H3.2 this residue is less methylated and, as predicted, acetylated to a higher extent than in histone H3.1 (Table II).

The higher levels of acetylation of histone H3.2 with an apparently wider pattern of accessibility are consistent with the concept that histone H3.2 with its higher acetylation levels is part of transcriptionally active chromatin. Such chromatin is expected to be partially unfolded with increased accessibility for proteins. This speculation suggests that the specific or preferential localization of histone H3.2 in activated chromatin causes its higher level of acetylation. It leaves open the question on the mechanism by which histone variants may become localized in different chromatin environments.

Histone methylation is generally considered to be an irreversible modification of lysines in soluble histones and in chromatin for which no function has been established (Thomas et al., 1975; Honda et al., 1975b; Duerre and Buttz, 1990). In histone H3, partial methylation and acetylation at the same lysines (residues 4 and 9) has been observed during sequencing of bovine and trout histone H3 (DeLange et al., 1972; Honda et al., 1975a). Some histone methylation has recently been shown to change in Drosophila when heat shock genes were induced or transcription was inhibited (Tanguay and Desrosiers, 1988; Desrosiers and Tanguay, 1989). Histone methylation may thus be important in the regulation of chromatin activity. However, it is unknown whether mono-, di-, and trimethylation are functionally identical. The distinct differences observed in the patterns for all three forms of lysine methylation (Table II) might suggest functional differences, but not enough is known for fruitful speculation. For this paper, it appears most important to realize that prior methylation of a lysine, especially of lysines 9 and 27, will limit the possibility for histone acetylation.

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REFERENCES

Alias, C. D., Richman, R., Gorovsky, M. A., Ziegler, Y. S., Touchstone, B., Bradley, W. A., and Cook, R. G. (1986) J. Biol. Chem. 261, 1041–1048

Ausio, J., Dong, F., and Van Holde, K. E. (1989) J. Mol. Biol. 206, 451–464

Braun, W. F., and von Holi, C. (1982) Eur. J. Biochem. 121, 501–510

Crane-Robinson, C., and Boehm, L. (1985) Biochem. Soc. Trans. 13, 303–306

DeLange, R. J., Hooper, J. A., and Smith, E. L. (1975) Proc. Natl. Acad. Sci. U. S. A. 69, 882–884

Desrosiers, R., and Tanguay, R. M. (1989) Biochem. Biophys. Res. Commun. 162, 1037–1043

Dimitrov, S. I., Apostolova, T. M., Makarov, V. L., and Pashov, I. G. (1986) FEBS Lett. 200, 322–326

Donahue, P. R., Palmer, D. K., Condie, J. M., Sabatini, L. M., and Blumenfeld, M. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 4744–4747

Duerre, J. A., and Buttz, H. R. (1990) in Protein Methylation (Paik, W. K., and Kim, S., eds) pp. 125–138, CRC Press, Boca Raton

Dumuis-Kervabon, A., Encontre, I., Etienne, G., Jauregui-Adell, J., Mery, J., Meunier, D., and Parello, J. (1986) EMBO J. 5, 1735–1742

Encztre, I., and Parello, J. (1988) J. Mol. Biol. 202, 673–676

Gross, D. S., and Garrard, W. T. (1987) Trends Biochem. Sci. 12, 293–297

Gross, D. S., and Garrard, W. T. (1988) Annu. Rev. Biochem. 57, 159–198

Halleck, M. S., and Gurley, L. R. (1982) Exp. Cell Res. 136, 271–280

Hobbes, T. R., Thorne, A. W., and Crane-Robinson, C. (1988) EMBO J. 7, 1395–1402

Honda, B. M., Dixon, G. H., and Candido, E. P. M. (1975a) J. Biol. Chem. 250, 8681–8685

Honda, B. M., Candido, E. P. M., and Dixon, G. H. (1975b) J. Biol. Chem. 250, 8686–8689

Jasinskiene, N. E., Jasinski, A. L., and Gineitis, A. A. (1985) Mol. Biol. Rep. 10, 199–203

Jasinskiene, N. E., Jasinski, A. L., and Gineitis, A. A. (1988) Mol. Biol. (Moscow) 22, 257–266

Marion, C., Roux, B., and Coulet, P. R. (1983) FEBS Lett. 157, 317–321

Matthews, H. R., and Waterborg, J. H. (1985) in The Enzymology of Post-translational Modification of Proteins (Freedman, R. B., and Hawkins, H. C., eds) Vol. 2, pp. 125–156, Academic Press, London

Pederson, D. S., and Simpson, R. T. (1988) ISV/Atlas Sci. Biochem. 1, 155–160

Pfeffer, U., Ferrari, N., Tosetti, F., and Vidal, G. (1986) Exp. Cell Res. 178, 25–30

Pfeffer, U., Ferrari, N., Tosetti, F., and Vidal, G. (1989) J. Cell Biol. 109, 1007–1014

Rill, R. L., and Oosterhof, D. K. (1982) J. Biol. Chem. 257, 14875–14880

Tanguay, R. M., and Desrosiers, R. (1988) Adv. Exp. Med. Biol. 231, 353–362

Thomas, G., Lange, H-W., and Henupel, K. (1970) Eur. J. Biochem. 51, 609–615

Turner, B. M., and Fellows, G. (1989) Eur. J. Biochem. 179, 131–170

Vanfleteren, J. R., Van Bun, S. M., and Van Beeumen, J. J. (1987) FEBS Lett. 211, 59–63

Vidal, G., Ferrari, N., and Pfeffer, U. (1988) Adv. Exp. Med. Biol. 231, 583–596

Von Holt, C., and Brandt, W. F. (1977) Methods Cell Biol. 16, 205–222

Waterborg, J. H. (1990) Electrophoresis, in press

Waterborg, J. H., Winicov, I., and Harrington, R. E. (1987) Arch. Biochem. Biophys. 256, 167–178

Waterborg, J. H., Harrington, R. E., and Winicov, I. (1989) Plant Physiol. 90, 237–245

Wu, S. C., Gyorgyey, J., and Dudas, D. (1989) Nucleic Acids Res. 17, 3057–3063
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