ever, provide no indication as to whether photolabeling occurs on only one subunit of the dimer, as could be expected for a half of the site mechanism, or whether both subunits of the dimer are covalently photolabeled.

Registry No. ATR, 17754-44-8; NAP_3-ATR, 83876-80-6; NAP_4-ATR, 83876-81-7; azidobenzoyl-ATR, 83876-82-8; adenine nucleotide translocase, 9068-80-8.

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Glycosylation, ADP-Ribosylation, and Methylation of *Tetrahymena* Histones†

Beatriz Levy-Wilson*

ABSTRACT: We have examined some of the postsynthetic modifications that occur in macronuclear histones from *Tetrahymena thermophila*. When purified macronuclei are incubated with [^{32}P]NAD^+, histones H1, H2A, H2B, and H3 are ADP-ribosylated. Furthermore, histones H1, H2A, H2B, and H3 contain fucose and mannose residues as evidenced by the incorporation of [^{3}H]fucose and by the specific binding to these proteins of gorse seed lectin and concanavalin A. Finally, our studies on incorporation of methyl groups into histones show that histone H2A, together with the related nonhistone protein A24, is methylated in *Tetrahymena*.

Histones are fundamental components of nucleosomes, the building blocks of chromatin structure in eucaryotes [for a review, see Kornberg (1977)]. Two each of histones H2A, H2B, H3, and H4 are associated with 140 base pairs of DNA to constitute the nucleosome core particle. The fifth histone, H1, is found bound to DNA in the internucleosomal "linker" region. Even though the bulk of the DNA in most eucaryotic cells is organized into nucleosomes, there is ample evidence that nucleosomes in transcriptionally active chromatin have a different structure from those in inactive chromatin. These differences include the presence in active nucleosomes of high mobility group proteins (HMG's) in stoichiometric amounts in relation to the inner histones (Hutcheon et al., 1980; Egan & Levy-Wilson, 1981), the lack of methylation of DNA sequences around active genes (McGhee & Ginder, 1979), and a high level of histone acetylation in transcriptionally active nucleosomes (Levy-Wilson et al., 1979; Nelson et al., 1980).

To further understand the structural prerequisites of transcriptionally active chromatin domains, we have examined the occurrence of a variety of postsynthetic modifications in hi-

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stones from *Tetrahymena thermophila*. In this organism transcriptionally active chromatin is absent from the germ-line micronucleus and occurs exclusively in the macronucleus. This latter nucleus is therefore a favorable subject for elucidation of the attributes of transcriptionally active chromatin.

Previous work by Gorovsky and co-workers demonstrated that all four *Tetrahymena* "core" histones, H2A, H2B, H3, and H4, are acetylated in the transcriptionally active macronuclei (Gorovsky et al., 1978; Vavra et al., 1982). Furthermore, histones H1, H2A, H3, and H2B are also phosphorylated in *Tetrahymena* (Gorovsky et al., 1978). We have extended this work by examing other modifications: ADP-ribosylation, glycosylation, and methylation.

Poly(ADP-ribose) is synthesized from its precursor NAD^+, by the chromatin-bound enzyme poly(ADP-Rib) synthetase (Ueda et al., 1975). Both in vivo and in isolated nuclei, ADP-ribose is incorporated into histones H2A, H1, and H3. The biological roles of poly(ADP-ribosylation) of nuclear proteins remains obscure. Possible functions include regulation of cellular growth (Ferris & Clark, 1971), DNA synthesis (Burzio & Koide, 1973), gene expression (Proctor & Casida, 1975), chromatin structure modification (Giri et al., 1978), activation or inhibition of RNA polymerases (Muller & Zahn, 1976), and DNA repair (Durkacz et al., 1980). The poly-(ADP-Rib) polymerase enzyme appears to be localized preferentially in nuclease-sensitive chromatin regions (Mullins et al., 1977) likely to correspond to the "active" domain (Weisbrod, 1982) or to newly replicated chromatin (Seale, 1975).

Glycosylation of chromosomal proteins has been a subject of active investigation for some time. Only recently has it been established that mammalian high mobility group (HMG) proteins are glycosylated (Reeves et al., 1981). Not much is known about glycosylation of histones. Because the glycosylated HMG proteins are associated with histones in a special subset of the nucleosomes, we ask whether histones were also susceptible to glycosylation reactions. *Tetrahymena* cells were chosen as a system due to their high levels of transcriptional activity.

Methylation of histones has been reported in many systems. In most cell types, the arginine-rich histones, H3 and H4, are methylated immediately after synthesis (DeLange et al., 1969; Honda et al., 1975). This methylation is stable and does not turn over during the lifetime of the histone.

We wished to investigate whether *Tetrahymena* histones are subject to a metabolically active type of methylation, in view of recent reports on "active" methylation of physiologically significant proteins (Wang et al., 1982).

Materials and Methods

Cell Culture and Fucose Labeling. Tetrahymena thermophila, strain BVII, were cultured axenically in 1% protease peptone as described by Gorovsky et al. (1978). Cells were labeled for 20 h with ^{3}H-labeled amino acids or [^{3}H]fucose (1 mCi/100 mL, 50 Ci/mmol) in media consisting solely of 10 mM Tris-HCl, pH 8.0 (starved conditions). In this medium, incorporation of amino acids into macromolecules remains linear for several hours.

Isolation of Nuclei. Nuclei were obtained from *Tetrahymena* cells by a modification of the procedure of Zaug & Cech (1980). Exponentially growing cells were recovered by centrifugation for 20 min at 5000 rpm in Sorvall GSA rotor. Subsequent steps were performed at 0 °C and all solutions employed contained the protease inhibitor PMSF (phenylmethanesulfonyl fluoride) at a final concentration of 1 mM. The cell pellet was resuspended in a solution of TMS (0.25 M sucrose–10 mM $MgCl_2$–3 mM $CaCl_2$–10 mM Tris-HCl, pH 7.5), at a density of 3×10^6 cells/mL. Nonidet P-40 (10% v/v) was added to a final concentration of 0.32%, and the cell suspension was agitated for about 1 h in a tissue homogenizer or until nuclei appeared to be free of cellular debris. At that point, crystals of sucrose were added slowly to 0.813 g/mL and allowed to dissolve by containing the homogenization for another 30–40 min on ice. Nuclei were then pelleted by centrifugation for 30 min at 10 000 rpm in a Sorvall SS-34 or SA-600 rotor. The nuclear pellets were combined and washed 2 or 3 times more with TMS prior to further use. This procedure, when applied to *Tetrahymena* GL cells, yields intact transcriptionally active macronuclei. When BVII cells are used, one obtains macronuclei in high yields (>80%) and variable amounts of transcriptionally inactive micronuclei.

Labeling of Nuclei with [^{32}P]NAD$^+$. Purified nuclei derived from exponentially growing cells were resuspended in a solution containing 0.25 M sucrose–10 mM Tris HCl (pH 8.2)–60 mM KCl–10 mM $MgCl_2$–4 mM NaF and incubated with 0.5 mCi of [^{32}P]NAD^+ (10–50 Ci/mmol) for 45 min at 10 °C. Nonradioactive NAD^+ was then added at a final concentration of 1 mM and labeling proceeded for another hour (Levy-Wilson, 1981b).

Labeling of Nuclei with [^{3}H]-S-Adenosylmethionine. Nuclei derived from 4×10^8 cells were resuspended in 20 mL of TMS and incubated for 1 h at 25 °C with 0.15 mCi of [^{3}H]-*S*-adenosylmethionine (15 Ci/mmol). β-Mercaptoethanol was also added at a concentration of 2 mM.

Histone Isolation. Histones were extracted from macronuclei that had been previously washed extensively with 0.35 M NaCl to remove HMG proteins. The washed nuclei were incubated at 4 °C for 1 h in 0.2 M H_2SO_4 with constant stirring. After centrifugation for 15 min at 10 000 rpm in the Sorvall HB-4 rotor, histones were precipitated from the acid-soluble supernatant by the addition of 3 volumes of 95% ethanol. The precipitate was repeatedly washed with 95% ethanol and lyophilized.

Polyacrylamide Gel Electrophoresis of Histones. Tetrahymena histones were analyzed by polyacrylamide gel electrophoresis in various systems. First, acid–urea gels containing or lacking Triton X-100 at a concentration of 0.22% were used as previously described (Egan & Levy-Wilson, 1981; Levy-Wilson, 1981a). Sodium dodecyl sulfate [$NaDodSO_4$ (SDS in the figures)] containing 10% gels were also run according to the procedure of Laemmli (1970). Finally, two-dimensional gels, consisting of a first-dimension Triton-containing acid–urea gel (TAU) and a second-dimension $NaDodSO_4$ gel were also used, as described by Allis et al. (1980), with some modifications. Conditions for staining of the gels and autoradiography of [^{32}P]orthophosphate-labeled or ^{125}I-containing samples were as previously described (Levy-Wilson et al., 1983). In some cases, labeled protein bands were sliced out of the gels, solubilized in 5% Protosol in Ominfluor–toluene scintillation mixture and counted.

Reactions of Histones with Lectins. Histones were electrophoresed on Triton-containing acid–urea gels or $NaDodSO_4$ gels as described above. The gels were stained with Coomassie blue R (2.5 g of dye–450 mL of methanol–450 mL of H_2O) for 30 min and destained in a mixture of H_2O–methanol–acetic acid (560:180:60 v/v). The gels were photographed and then neutralized by soaking in many changes of 20 mM sodium phosphate, pH 7.0. Once the gels were equilibrated in this solution (50 mL), a solution of (12×10^7 cpm) ^{125}I-labeled concanavalin A was added and incubation continued for 1–2 h at room temperature with constant shaking. Half of the gel

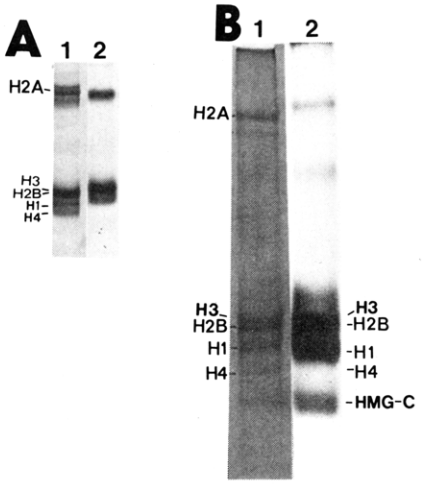

FIGURE 1: ADP-ribosylation of *Tetrahymena* histones. Histones were isolated from nuclei (labeled with [^{32}P]NAD$^+$) that had been first extracted with 0.35 M salt to remove HMG's. The acid-soluble proteins were electrophoresed on Triton-containing polyacrylamide gels. Gels were stained for 30 min with Coomassie blue, destained, photographed and autoradiographed either wet at 4 °C or dry at −70 °C for various time periods. Slot A_1 shows the stained portion of one such gels; slot A_2 is the corresponding autoradiogram. Slot B_1 is the stained portion of a gel from a different labeling experiment; slot B_2 is the corresponding autoradiogram.

was incubated under the same conditions but also in the presence of 0.1 M methyl α-D-glucopyranoside and 0.1 M methyl α-D-mannopyranoside. Other gels were incubated in 100 mL in the presence of 2.5×10^7 cpm of ^{125}I-labeled gorse seed lectin from *Ulex europaeus* and 50% ethylene glycol with or without 0.2 M L-fucose as a competitor. After incubation with the iodinated lectins, the gels were washed extensively with various changes of 20 mM sodium phosphate, pH 7.0, solution to reduce the background. The gels were then dried and exposed with X-ray film at −70 °C for the desired periods of time. The iodination of both lectins and the conditions for incubation of the gels with the lectins were as described by Rostes et al. (1977). $NaDodSO_4$ gel electrophoresis of the iodinated gorse seed and Con A lectins, followed by autoradiography, showed one band of labeled protein in each case, comigrating with the corresponding unlabeled standard lectins.

Snake Venom Phosphodiesterase Digestion of ADP-Ribosylated Histones. Labeled histones (100 μg) were digested with snake venom phosphodiesterase (Boehringer Mannheim) (10 μg) in a final volume of 20 μL in a solution also containing 2 μL of Tris-HCl, pH 8.0 (1 M) and 0.3 μL of $MgCl_2$ (1 M). Incubation was for 2 h at 37 °C. After incubation, some samples were precipitated with 10% trichloroacetic acid, filtered onto glass fiber filters, and counted. Other samples were run on gels in parallel with untreated samples and then autoradiographed.

Results

ADP-Ribosylation of Tetrahymena Histones. ADP-ribosylated histones were isolated from nuclei that had been labeled with [^{32}P]NAD$^+$ and electrophoresed on TAU gels. Figure 1 shows the results of two labeling experiments using different batches of nuclei. Both experiments show that histones H2A, H2B, H3, and H1 are ADP-ribosylated. That the labeled groups incorporated into histones indeed correspond to ADP-ribose groups was shown by their sensitivity to snake venom phosphodiesterase and to 0.1 M NaOH (not shown). Because the resolution between the five histones was incomplete in this one-dimensional gel, we resorted to two-dimensional gels to unequivocally identify all of the labeled histones.

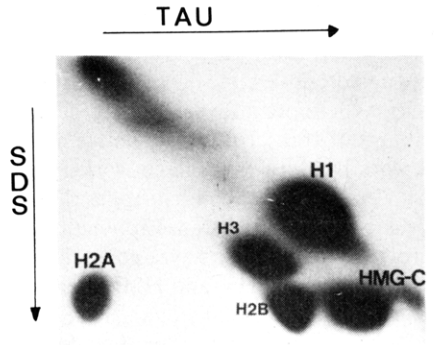

FIGURE 2: Two-dimensional polyacrylamide gel electrophoresis of ADP-ribosylated histones. A sample containing acid-soluble proteins, derived from ADP-ribosylated nuclei, was analyzed on a two-dimensional polyacrylamide gel. The figure represents the autoradiogram of the labeled proteins separated on the gel: TAU, Triton–acid–urea (first dimension); SDS, sodium dodecyl sulfate (second dimension).

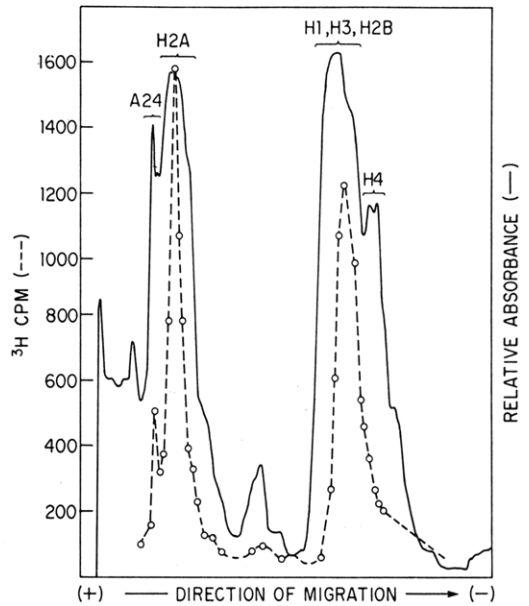

FIGURE 3: Incorporation of [^{3}H]fucose into *Tetrahymena* histones. Exponentially growing *Tetrahymena* BVII cells were labeled with [^{3}H]fucose (1 mCi/100 mL of culture), in 10 mM Tris, pH 8.0, for 16 h at 29 °C. A normal 2-L culture was grown in parallel to provide unlabeled carrier proteins. Nuclei were isolated and histones extracted from them. Aliquots, containing ~50–100 μg of protein, were electrophoresed in TAU slab gels. The figure shows a densitometer scan of the stained proteins, together with the distribution of tritiated counts.

Figure 2 illustrates the autoradiogram corresponding to one such gel. Four of the five histones, namely, H2A, H2B, H1, and H3, are ADP-ribosylated but H4 is not modified. One of the HMG proteins, HMG-C, coextracts with the histones and it is also ADP-ribosylated (B. Levy-Wilson et al., unpublished experiments).

Are Tetrahymena Histones Glycoproteins? To investigate whether *Tetrahymena* histones possess sugar residues covalently attached to the polypeptide backbone, we first chose to study the incorporation of [^{3}H]fucose. Fucose was chosen because it is one of the few sugars that is not fermented by *Tetrahymena* cells (Hill, 1972); therefore, it is likely that incorporation of radioactivity from labeled fucose into proteins represents glycosylation. Cells were labeled under starved conditions with L-[^{3}H]fucose and histones were purified from nuclei. Figure 3 shows the electrophoretic profile of L-[^{3}H]-fucose labeled histones. The radioactivity incorporated into

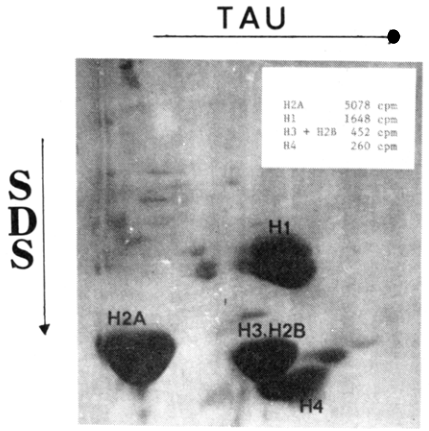

FIGURE 4: Two-dimensional gel electrophoresis of the [^{3}H]fucose-labeled histones. A sample equivalent to that in Figure 3 was analyzed on two-dimensional polyacrylamide gels. Each one of the stained spots was sliced from the gel; the proteins were solubilized by incubation for 16 h in an oven at 60 °C in an Omnifluor scintillation mixture containing 5% Protosol. The solubilized counts were then assayed in a scintillation counter.

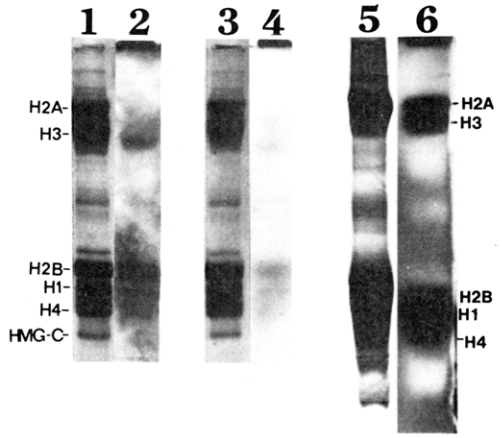

FIGURE 5: Binding of fucose-specific gorse seed lectin to *Tetrahymena* histones. Samples containing some 100–150 μg of *Tetrahymena* histones were electrophoresed on TAU gels and incubated with iodinated gorse seed lectin, as described under Materials and Methods. Two identical samples of unlabeled *Tetrahymena* histones were electrophoresed in parallel in the same gel. These two gel slices were incubated with ^{125}I-labeled gorse seed lectin, either in the absence (slot 1) or presence (slot 3) of 0.2 M fucose. Slots 1 and 3 represent stained portions of these two samples. Slot 2 is the autoradiogram corresponding to the sample in slot 1. Slot 4 is the autoradiogram corresponding to the sample in slot 3. In slots 5 and 6 we show the results of another such experiment. Slot 5 is the stained portion of the gel; slot 6 is the corresponding autoradiogram.

histones appeared to be associated with all histone species with the possible exception of H4. This preliminary result encouraged us to determine which histones incorporated fucosyl residues by resolving the various histones on two-dimensional gels as illustrated in Figure 4. Histone H2A incorporated the highest amount of counts, followed by histones H1, H4, H3, and H2B. Thus, all five *Tetrahymena* histones appear to contain L-fucose.

If histones indeed contain fucosyl residues, they should bind to the fucose-specific, gorse seed lectin. Furthermore, binding of the lectin to histone should be competed for by an excess of L-fucose. These predictions were tested experimentally and the results are illustrated in Figure 5. In these lectin binding experiments, H3 was converted to its oxidized form to facilitate separation from the other histone species. The first four slots show one experiment in which histones have been presented with iodinated gorse seed lectin, both in the presence (slots

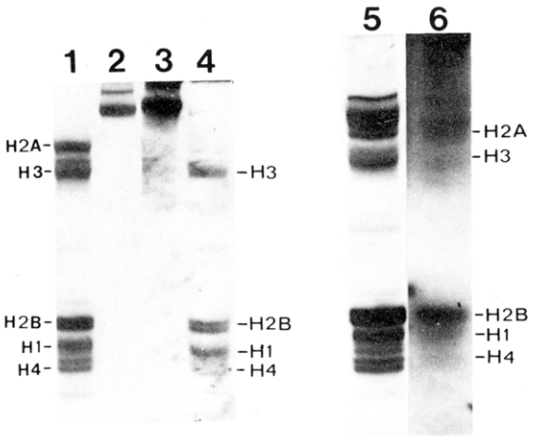

FIGURE 6: Binding of iodinated concanavalin A to *Tetrahymena* histones. Samples containing some 100–300 μg of *Tetrahymena* histones were electrophoresed on TAU gels as described under Materials and Methods. Horseradish lactoperoxidase, a standard mannose-containing glycoprotein, was electrophoresed in parallel in the same gel. The gel slices were incubated with ^{125}I-labeled Con A, followed by autoradiography. Slot 1 is the stained portion of a gel of *Tetrahymena* histones; slot 4 is the corresponding autoradiogram. Slot 2 shows a stained lane of lactoperoxidase; slot 3 is the corresponding autoradiogram. Slot 5 illustrates a different, larger sample of *Tetrahymena* histones treated with the lectin; slot 6 is the corresponding autoradiogram.

3 and 4) and in the absence (slots 1 and 2) of 0.2 M fucose. The autoradiogram in slot 2 shows radioactive bands associated with histones H3, H2B, and more faintly H1 and H4. The intensity of these bands is reduced considerably in the presence of L-fucose (slot 4). Because the amount of some of the proteins was insufficient to give strong bands, we performed another series of experiments with overloaded gels, in order to detect any additional binding of the lectin by the histones. The results of one such experiment are shown in slots 5 and 6. The autoradiogram (slot 6), clearly shows strong binding of gorse seed lectin to histones H2A, H3, H2B, and H1 and weak binding to H4. It is difficult to ascertain if histone H4 does contain fucose residues. The reactions with gorse seed lectin are quite faint; however, judging by the data obtained from the two-dimensional gels, we find that H4 appears to incorporate some L-[^{3}H]fucose. Therefore, we conclude that *Tetrahymena* histones are fucose containing glycoproteins.

We then asked whether these histones can bind a second lectin, concanavalin A, whose preferred sugar recognition site in proteins is mannose. Binding was assayed in a manner analogous to that employed above with gorse seed lectin. The results, illustrated in Figure 6, show that *Tetrahymena* histones do indeed bind to Con A, suggesting that they contain mannose residues. All five histones appear to bind the lectin, with different intensities. The binding of Con A to each of the histones was greatly diminished in the presence of the competitor sugars methyl α-mannoside and α-glucopyranoside at a concentration of 0.2 M. The validity of the gel binding assay and the competition reaction was checked with lactoperoxidase, a glycoprotein known to contain mannose residues.

Histone Methylation in Tetrahymena. To ascertain whether *Tetrahymena* histones are methylated, we labeled purified nuclei with [^{3}H]-*S*-adenosylmethionine, followed by histone isolation and electrophoresis on TAU gels. In Figure 7, we observe that H2A appears to be the only methylated histone. Interestingly, the nonhistone protein A_{24} is also methylated under these same conditions but none of the other histones appear to incorporate [^{3}H]methyl label. To further check the validity of these results, we analyzed a sample of [^{3}H]histones derived from a different methylation experiment in two-di-

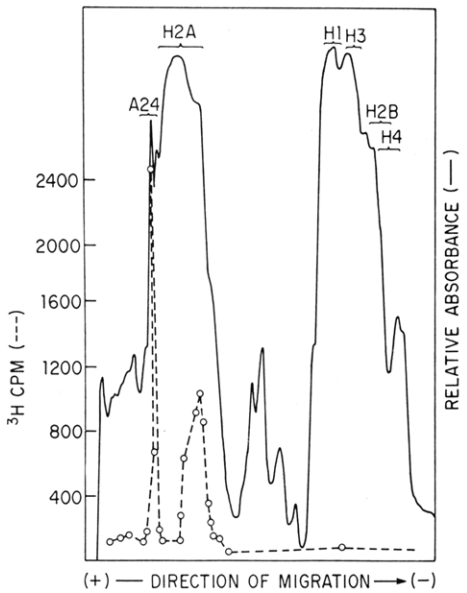

FIGURE 7: Incorporation of [^{3}H]-*S*-adenosylmethionine into *Tetrahymena* histones. Purified *Tetrahymena* nuclei were labeled with [^{3}H]-*S*-adenosylmethionine as described under Materials and Methods. Histones were isolated and electrophoresed on TAU gels. Gels were sliced into 1-mm slices, and the protein-associated radioactivity was solubilized and counted.

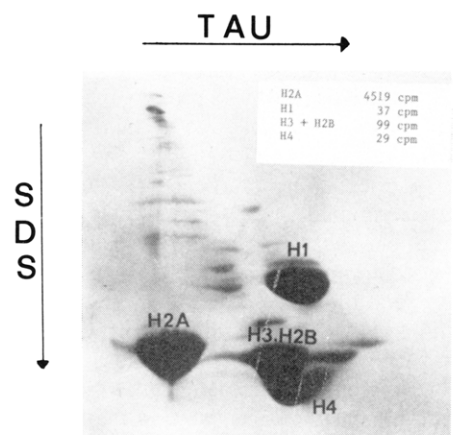

FIGURE 8: Two-dimensional electrophoresis of methylated histones. Aliquots of [^{3}H]methylated labeled histones were separated on two-dimensional gels. Individual stained spots were solubilized and counted as described under Materials and Methods.

mensional gels (see Figure 8). Only H2A was methylated.

Discussion

We have examined the distribution of ADP-ribose groups in histones from *Tetrahymena* (BVII) nuclei. Our results (Figure 1 and 2) show that histones H2A, H2B, H1, and H3 incorporate radioactive ADP-ribose groups when nuclei are incubated with [^{32}P]NAD$^+$. H4 does not appear to be modified in this manner. Our data is in good agreement with similar results in various other organisms, such as mammals (Sugimura, 1973) and fish (Levy-Wilson, 1981b). At present, the functional significance of this type of modification of histone remains poorly understood. Several hypotheses have been postulated to explain these results. For instance, one could argue that, ADP-ribosylation of histones, by diminishing the protein's overall positive charge, may loosen the interaction between histones and DNA in the vicinity where the modification occurred. This would leave some DNA sequences more exposed to the action of RNA polymerases with a consequent increase in transcriptional activity. Our results concerning histone ADP-ribosylation of the trancriptionally active *Tetrahymena* macronuclei are certainly consistent with that hypothesis. On the other hand, an analogous argument has been used by other investigators (Jump et al., 1979) to promote a role of histone ADP-ribosylation in DNA replication; i.e., a loosening of the interactions between histones and DNA would favor DNA polymerase action. A third idea has been put forward by Shall and co-workers, who postulated that ADP-ribose plays a role in the cellular recovery from DNA damage. According to this theory, in order to maintain DNA repair at high efficiency, the cell needs to maintain high levels of activity of ADP-Rib polymerase, the enzyme that performs the modification reaction (Durkacz et al., 1980). In the case of H1, ADP-ribosylation may lead to chromosome condensation, perhaps via cross-linking of several ADP-ribosylated H1 molecules (Stone et al., 1977).

Our results on the glycosylation of histones were unexpected and may shed light upon the relationships between chromatin structural transitions and genetic activity. It is clear both from incorporation studies and from the gorse seed binding data that *Tetrahymena* histones contain fucosyl residues attached to them. Furthermore, the binding of concanavalin A suggests that, in addition to fucose residues, histones also contain mannose moieties. This would put histones in the category of glycoproteins.

It is not possible to obtain an accurate estimate of the fucose content of individual histones because the conditions of isotope uptake would not achieve random labeling and the pool size is unknown. However, from the extent of fucose incorporation and its specific radioactivity, from the approximate molecular weight of the histones, and assuming a 5% counting efficiency of the ^{3}H-labeled gel slices dissolved in Protosol, we can estimate that a minimum of one in a thousand nucleosomes contain a fucosylated H2A molecule. If the residues are stable and only a small proportion of the histones are labeled under starvation conditions, the actual number could be much higher. If only a minor fraction of the nucleosomes are modified in this manner, they may differ from bulk nucleosomes in their intranuclear localization, perhaps in their proximity to the nuclear membrane.

It is not known yet whether these glycosylation reactions take place in the rough endoplasmic reticulum, in the vicinity of the polysomes engaged in histone synthesis, in the nuclear membrane, or within the nucleus. It is known, nevertheless, that fucose-specific glycosyltransferases can transfer a sugar residue to the hydroxyl group of amino acids such as serine and threonine (Klinger et al., 1981). All five *Tetrahymena* histones contain both these residues (Johmann & Gorovsky, 1976). From the relative intensities of the autoradiographic bands obtained upon binding of ^{125}I-labeled Con A to histones and horseradish lactoperoxidase, and taking into account the differences in size between the lactoperoxidase and histones, it appears that the mannose content of H3 and H2B are about 50 times lower than that of the standard protein.

To date, we do not know whether glycosylation of histones is confined to the *Tetrahymena* transcriptionally active macronucleus or whether it is a common occurrence in histones of other eucaryotes. We were fortunate in being able to use fucose in our incorporation studies, a sugar that is not fermented by the cells (Hill, 1972). This may be more difficult to achieve in mammalian cells. However, the lectin binding studies should be applicable to most organisms. *Tetrahymena* macronuclei are unusual in that a very high proportion of the genome is transcribed (Stathopoulos et al., 1980). If the glycosylation reaction is correlated with the degree of tran-

scriptional activity, it may be more difficult to measure in nuclei of mammalian cells, where the proportion of the genome that is transcribed is much lower. This may be indeed the reason why this phenomenon has not been reported hitherto. The possible correlation between ADP-ribosylation and glycosylation of histones with transcriptional activity can, in principle, be tested in *Tetrahymena*, by comparison of the degree of modification of macronuclear vs. micronuclear histones. This, of course, would require pure nuclei.

We found that H2A is the only histone methylated under the conditions used. In addition, the nonhistone protein A_{24} (composed of H2A linked to ubiquitin) (Goldknopf & Busch, 1977) was also methylated. It has been known for some time that the bulk of histone methylation in higher cells occurs in the arginine-rich histones H3 and H4 (DeLange et al., 1969). This modification is metabolically stable (Honda et al., 1975) and, thus, difficult to detect in incorporation studies such as the one described here. To date, the only case in which H2A has been found to be methylated is that of the regenerating pancreatic epithelium. In this case, methylation of H2A (and H1) occurred 3 days prior to DNA synthesis (Marsh & Fitzgerald, 1973). These results suggest that the methylation of lysine-rich histones, such as H2A, may be characteristic of tissues with high metabolic activity and thus its role may differ from methylation of the arginine-rich histones. At present, we do not know the turnover rate of methyl groups in H2A. However, methyl transferases and demethylases are known to coexist in nuclei from various organisms (Paik & Kim, 1980).

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