Loeb, L. A., Kunkel, T. A., & Schaaper, R. M. (1980) in *Mechanistic Studies on DNA Replication and Genetic Recombination*, pp 735–751, Academic Press, New York.
Margison, G. P., & O'Connor, P. J. (1973) *Biochim. Biophys. Acta 331*, 349–356.
McHenry, C. S., & Crow, W. (1979) *J. Biol. Chem. 254*, 1748–1753.
Meyer, R. R., Glassberg, J., & Kornberg, A. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 1702–1705.
Sanger, F., Nicklen, S., & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5463–5467.
Schaaper, R. M., & Loeb, L. A. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 1773–1777.
Schaaper, R. M., & Glickman, B. W. (1982) *Mol. Gen. Genet. 185*, 404–407.
Schaaper, R. M., Glickman, B. W., & Loeb, L. A. (1982) *Mutat. Res. 106*, 1–9.
Schaaper, R. M., Kunkel, T. A., & Loeb, L. A. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 487–491.
Shearman, C. W., & Loeb, L. A. (1979) *J. Mol. Biol. 128*, 197–218.
Singer, B. (1976) *Nature (London) 264*, 333–339.
Sinha, N. K., & Haimes, M. D. (1981) *J. Biol. Chem. 256*, 10671–10683.
Strauss, B., Scudiero, D., & Henderson, E. (1975) in *Molecular Mechanisms for Repair of DNA*, Part A, pp 13–24, Plenum Press, New York.
Strauss, B., Rabkin, S., Sagher, D., & Moore, P. (1982) *Biochimie 64*, 829–838.
Villani, G., Boiteux, S., & Radman, M. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 3037–3041.
Weissbach, A. (1977) *Annu. Rev. Biochem. 46*, 25–47.
Weymouth, L. A., & Loeb, L. A. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 1924–1928.
Witkin, E. M. (1976) *Bacteriol. Rev. 40*, 869–907.
Zakour, R. A., & Loeb, L. A. (1982) *Nature (London) 295*, 708–710.

Relationship between Histone H1 Poly(adenosine diphosphate ribosylation) and Histone H1 Phosphorylation Using Anti-Poly(adenosine diphosphate ribose) Antibody†

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ABSTRACT: The chromatin-associated enzyme poly(ADP-Rib) polymerase catalyzes the posttranslational modification of histones. Antibody to poly(ADP-Rib) has been coupled to Sepharose, and the resultant immunoadsorbent was used to fractionate, specifically, histone H1 subpopulations undergoing this nuclear protein modification. When this method of separation was used, it was additionally observed that poly(ADP-ribosylated) H1 species were highly accessible to in vitro phosphorylation by nuclear protein kinase. Phosphorylated H1 molecules were retained by the anti-poly(ADP-Rib)–Sepharose column due to the presence of endogenous poly(ADP-Rib) components. Degradation of the latter moieties on phosphorylated H1 reversed their adsorption to the column.

Histone H1 has been demonstrated to play a major role in the higher ordered structure of chromatin (Worcel, 1978; Thoma & Koller, 1977). Multiple forms of histone H1, in tissues and cells, differ in their primary structure. In addition, postsynthetic modifications of histone H1 such as poly(ADP-ribosylation) and phosphorylation are thought to modulate the regulation of various biological processes. Our laboratory has been particularly interested in the poly(ADP-ribosylation) of histone H1 (Nolan et al., 1980; Butt et al., 1980; Wong et al., 1982, 1983). With the recent development of a highly specific antibody directed against the modified moiety, poly(ADP-Rib),[1] we thought it timely to test whether an immunological affinity method could be employed to bind selectively those very limited subpopulations of histone H1 within chromatin which undergo ADP-ribosylation. Second, it was of importance to ascertain whether or not phosphorylation and poly(ADP-ribosylation) of histone H1 are coordinated within the cell.

Poly(ADP-Rib) polymerase, a chromatin-associated enzyme, catalyzes the successive transfer of the ADP-Rib moiety of NAD to various nuclear proteins. One novel product of this reaction is an H1 complex, consisting of two molecules of H1 cross-linked by 15–16 units of poly(ADP-Rib) (Stone et al., 1977). We have speculated that this poly(ADP-Rib) complex of H1 may help stabilize nucleosomes during various physiological events of the cell cycle (Wong et al., 1983). From reconstitution studies, poly(ADP-ribosylated) H1 complex has been indirectly shown to be involved in the condensation of those limited domains of chromatin undergoing this modification (Butt et al., 1980; Wong et al., 1982).

Kidwell & Mage (1976) have shown that the maximal accumulation of cellular poly(ADP-Rib) occurs during the G2 phase of the eukaryotic cell cycle; recent immunological data have also suggested very high activity for the enzymatic synthetic reaction during metaphase of the cell cycle (Tanuma

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[1] Abbreviations: ADP-Rib, adenosine diphosphate ribose; NAD, nicotinamide adenine dinucleotide; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane; KSCN, potassium thiocyanate; Cl_3CCOOH, trichloroacetic acid; ATP, adenosine 5′-triphosphate; cAMP, adenosine cyclic 3′,5′-phosphate; PCA, perchloric acid; DTT, dithiothreitol.

0006-2960/83/0422-2384$01.50/0

& Kanai, 1982). We have recently presented data, using the poly(ADP-Rib) antibody, and proteins extracted directly from living cells to confirm the natural occurrence of H1 poly(ADP-Rib) complex in living cells; this could only be readily demonstrated in vivo when cells were synchronized at the G2 phase of the cell cycle (Wong et al., 1983). In this regard, it is of interest that hyperphosphorylation of H1 occurs during the same phases of the cell cycle as poly(ADP-ribosylation) and chromatin condensation (Ajiro et al., 1981a,b).

In the experiments presented below, we have first investigated whether an anti-poly(ADP-Rib)–Sepharose immunoabsorbent could be utilized to isolate selectively poly(ADP-ribosylated) species of histone H1. Subsequently, the immunofractionation characteristics of samples of H1 containing both phosphorylated and poly(ADP-ribosylated) species of this histone were examined.

Materials and Methods

HeLa Cell Growth and Preparation of Nuclei. HeLa S_3 cells were maintained in suspension cultures at 37 °C in Spinner flasks. All the cells used in the present studies were grown asynchronously to a mid-log density of 8×10^5 cells/mL culture medium.

HeLa nuclei were prepared by a procedure which included a Triton X-100 wash step according to Sporn et al. (1969).

Assay for Nuclear Kinase and Poly(ADP-Rib) Polymerase. HeLa nuclei were incubated with 0.25 M sucrose, 50 mM Tris-HCl, pH 8, 10 mM DTT, 10 mM $MgCl_2$, 18 μM [γ-^{32}P]ATP, and/or 50 μM [^{3}H]NAD for 5 min at 37 °C (Mullins et al., 1977). The reactions were terminated by the addition of 20% trichloroacetic acid and 5 mM pyrophosphate at 4 °C. Acid-insoluble material was collected on Whatman GF/C filter disks, and the radioactivity was determined in a liquid scintillation counter (Mullins et al., 1977).

Immunological Detection of Poly(ADP-Rib) Species with H1 and Poly(ADP-Rib) Antibodies. Nuclei were incubated with 100 μM [^{32}P]NAD as previously described (Nolan et al., 1980). Electrophoretic transfer of modified histone H1 from acetic acid/urea/polyacrylamide gel to nitrocellulose and the immunological detection of these proteins were performed as described by Wong et al. (1983). Anti-histone H1 IgG was a gift from Dr. M. Bustin.

Preparation of Modified Histone H1 for Poly(ADP-Rib) Antibody Column. HeLa nuclei were incubated with 0.25 M sucrose, 50 mM Tris-HCl, pH 8, 10 mM DTT, 10 mM $MgCl_2$, 18 μM [γ-^{32}P]ATP, and/or 50 μM [^{3}H]NAD for 10 min at 37 °C (Nolan et al., 1980; Rickwood et al., 1973). Subsequently, PCA-extracted histone H1 was loaded onto a polymer antibody column prepared as described by Malik et al. (1983). The column was washed in 1-mL fractions with phosphate-buffered saline. After the column was washed with 30 mL of saline, the bound material was eluted with 1.6 M KSCN. Aliquots were obtained from the unbound and the bound fractions to determine the acid-precipitable radioactivity.

For gel analysis of the labeled histone H1 present in the unbound and bound fractions, the protein was precipitated with 20% trichloroacetic acid and washed with acetone prior to polyacrylamide electrophoresis on an acetic acid/urea system.

Results

Specificity of Antibodies for Poly(ADP-ribosylated) Histone H1. The initial intention of this study was to develop techniques, utilizing poly(ADP-Rib) antibody coupled to Sepharose, to purify selectively poly(ADP-ribosylated) species of histone H1. These may represent 5% or less of the total nuclear pool of histone H1 at any one time (Nolan et al., 1980). To test the immunoreactivity of the poly(ADP-Rib) antibody toward the polymer bound to H1, HeLa cell nuclei were incubated with 100 μM *nonradioactive* NAD, and histone H1 was extracted selectively with 5% perchloric acid and separated by acetic acid/urea/polyacrylamide gel electrophoresis. Under these incubation conditions we have previously demonstrated that H1 is sequentially modified by progressively longer chains of poly(ADP-Rib) to yield 14 intermediates and ultimately a cross-linked H1 complex (Nolan et al., 1980). The gel in Figure 1 was subsequently blotted to nitrocellulose and incubated with either anti-poly(ADP-Rib) IgG (lane 1) or histone H1 antisera (lane 2), followed by ^{125}I-protein A. The data in lane 1 show that most of the poly(ADP-ribosylated) species of histone H1, including the complex, could be detected by the poly(ADP-Rib) antibody. The poly(ADP-Rib) antibody has been shown previously (Kanai et al., 1974) not to bind to mono(ADP-Rib) (i.e., lowest potential modified protein band in lane 1).

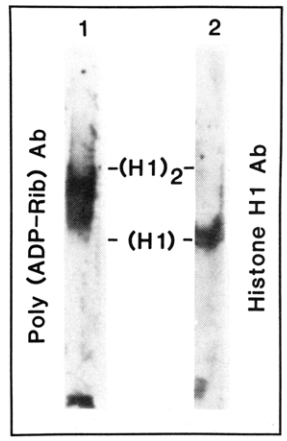

FIGURE 1: Immunological detection of poly(ADP-ribosylated) H1 with poly(ADP-Rib) and H1 antibodies. HeLa nuclei (1×10^8) were incubated with 100 μM nonradioactive NAD for 15 min at 25 °C as previously described (Wong et al., 1983). Histone H1 was selectively extracted, subjected to acetic acid/urea/polyacrylamide gel electrophoresis, transferred electrophoretically to nitrocellulose (Wong et al., 1983), and incubated with either anti-poly(ADP-Rib) IgG fraction (lane 1) or H1 antisera (lane 2) and ^{125}I-protein A as described in Wong et al. (1983). The blot was exposed for autoradiography.

The presence of histone H1 in the preparation is demonstrated in lane 2 (Figure 1). Clearly, only a small percentage of the H1 species becomes ADP-ribosylated under these conditions. However, by the use of more concentrated anti-H1 IgG than employed in the present study, the immunological presence of histone H1 was earlier demonstrated with all the modified species, including H1 complex, shown in lane 1 (Wong et al., 1983).

Selective Affinity of Poly(ADP-ribosylated) H1 by Anti-Poly(ADP-Rib)–Sepharose. In the experiment performed in Figure 2, nuclei were incubated with 50 μM [^{32}P]NAD and selectively extracted for histone H1 (Nolan et al., 1980). Electrophoretic analysis of this sample (Figure 3, lane 1) indicated the presence of the typical family of sequentially poly(ADP-ribosylated) H1 molecules, including H1 complex. A small quantity of contaminating poly(ADP-ribosylated) core histones and HMG proteins were also present. This sample was applied to an anti-poly(ADP-Rib)–Sepharose column, prepared as described by Malik et al. (1983). The column was eluted with 1-mL fractions of phosphate-buffered saline; a small flow-through peak of acid-insoluble radioactivity was noted in fractions 5–10. In addition (see below and Figure

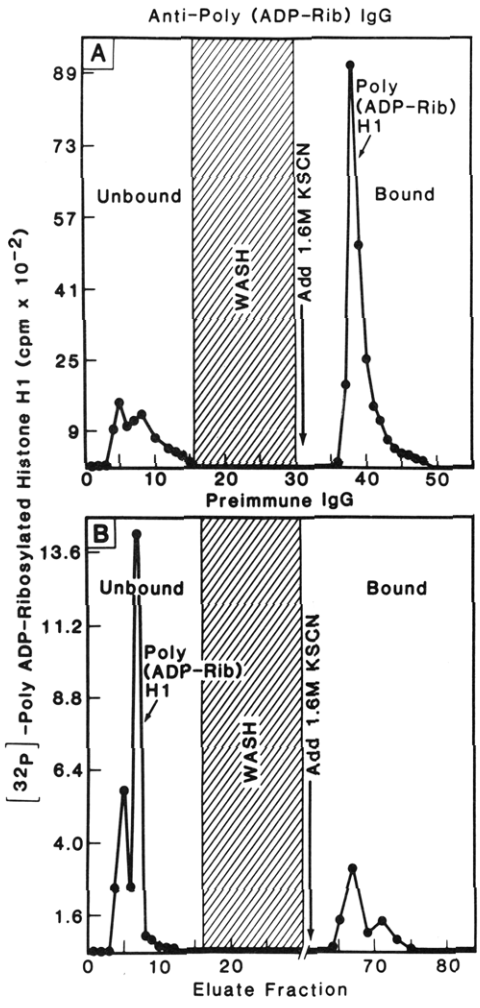

FIGURE 2: Selective retention of poly(ADP-ribosylated) histone H1 by anti-poly(ADP-Rib) IgG–Sepharose. Nuclei (1×10^8) were incubated with 50 μM [^{32}P]NAD (20 μCi), and poly(ADP-ribosylated) H1 was selectively extracted as described under Materials and Methods. The sample was subsequently applied to either anti-poly(ADP-Rib) IgG-Sepharose 4B (A) or preimmune IgG–Sepharose 4B (B). The column was washed with 14 1-mL fractions of phosphate-buffered saline. After the column was washed with 30 mL of saline, the bound material was eluted with 1.6 M KSCN. The amount of radioactivity incorporated into acid-insoluble material was determined in selected fractions (Mullins et al., 1977).

2B), the bulk of the unmodified H1 sample was eluted in these early fractions.

The column was subsequently washed extensively with fifteen 1-mL volumes of phosphate-buffered saline (fractions 15–29). Past experiments had indicated that specifically bound poly(ADP-ribosylated) nucleosomes could be effectively released from the antibody column with 1.6 M KSCN (Malik et al., 1983); accordingly this agent was used as an eluant commencing at fraction 30. An acid-insoluble radioactive peak was noted in fractions 36–42 which was subsequently (Figure 3) identified as poly(ADP-ribosylated) histone H1 by electrophoretic analysis.

As a control, the ^{32}P-labeled poly(ADP-ribosylated) H1 fraction was chromatographed on a preimmune IgG–Sepharose column (Figure 2B). In contrast to the selective retention noted in Figure 2A, essentially the entire sample passed directly through the column, with only minimal nonspecific binding.

Characterization of Immunopurified H1. Immunofractionated H1 samples from the unbound and bound fractions of Figure 2A were analyzed for histone H1 composition by

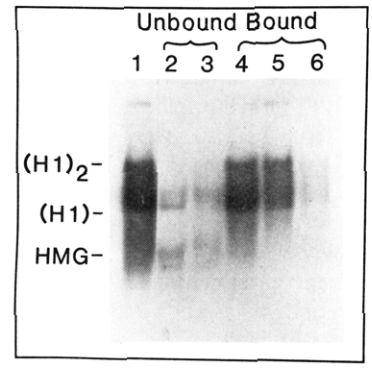

FIGURE 3: Analysis of poly(ADP-ribosylated) H1 species bound and unbound by anti-poly(ADP-Rib) IgG–Sepharose 4B immunofractionation. Samples from the unbound fractions 4–6 (lane 2), fractions 7–8 (lane 3) of unbound material, and fractions 37–38 (lane 4), 39–40 (lane 5), and 41–42 (lane 6) of bound material in Figure 2 were precipitated with 20% Cl_3CCOOH and washed with acidified acetone/acetone. Electrophoresis of the sample on an acetic acid/urea/polyacrylamide gel and autoradiography were performed as described under Materials and Methods. Lane 1 represents the original, unfractionated sample applied to the column in Figure 2.

Table I: Conditions Favoring Phosphorylation and Poly(ADP-ribosylation) in HeLa Cell Nuclei[a]

incubation conditions	[^{3}H]poly-(ADP-Rib) (dpm)	^{32}P phosphorylation (dpm)
[γ-^{32}P]ATP		45 000
[γ-^{32}P]ATP + 10 μM cAMP		70 000
[^{3}H]NAD	28 841	
[^{3}H]NAD + 10 μM cAMP	19 413	
[γ-^{32}P]ATP + [^{3}H]NAD	32 624	44 486
[γ-^{32}P]ATP + [^{3}H]NAD + cAMP	24 999	49 612

[a] Nuclei (1×10^7) were incubated with either 0.1 μCi of [^{3}H]NAD (50 μM) and/or 1 μCi of [γ-^{32}P]ATP (18 μM) at 37 °C for 5 min as described under Materials and Methods. The amount of radioactivity incorporated into the acid-insoluble fraction was determined.

precipitating the proteins with 20% trichloroacetic acid and performing acetic acid/urea/polyacrylamide gel electrophoresis and autoradiography (Figure 3). The specifically bound ^{32}P-labeled samples (lanes 4–6) contained a series of poly(ADP-ribosylated) H1 species containing progressively longer polymer chains and extending to the H1 poly(ADP-Rib) complex position in the gel.

Mono(ADP-ribosylated) H1 might not be expected to bind readily to the antibody column, based upon the past observation that ADP-ribose does not compete with poly(ADP-Rib) for antibody binding (Kanai et al., 1974). It was therefore of interest that the minimal quantity of radioactivity observed not to bind to the antibody column (fractions 4–8, Figure 2A) appeared to be predominately H1 species with very short $(ADP\text{-}Rib)_n$ units (lanes 2 and 3). In addition, two other 5% perchloric acid soluble bands, possibly mono(ADP-ribosylated) HMG proteins, present in small quantities in the unfractionated sample (lane 1), were not adsorbed by the antibody column.

The above experiments indicated that poly(ADP-ribosylated) species of histone H1 could be selectively examined by the immunoaffinity technique and further suggested that the method might be expanded toward studying the relationship between histone H1 phosphorylation and poly(ADP-ribosylation).

Phosphorylation of H1 in Vitro. Preliminary experiments were performed to establish an in vitro method for simulta-

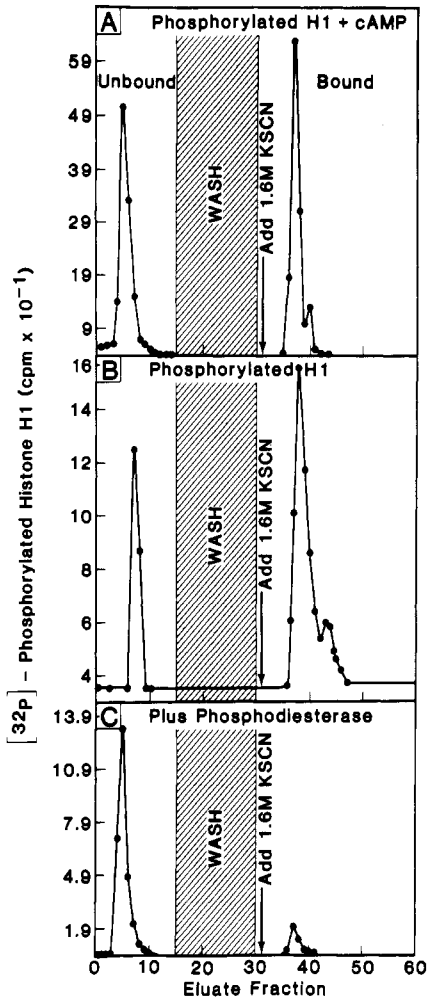

FIGURE 4: Fractionation of phosphorylated H1 on anti-poly(ADP-Rib) IgG–Sepharose. (A) Nuclei (2×10^8) were incubated with 25 μCi of [γ-^{32}P]ATP and 10 μM cAMP for 10 min at 37 °C as described under Materials and Methods. Histone H1 was selectively extracted with 5% perchloric acid and analyzed on the poly(ADP-Rib) antibody column as described in Figure 2. (B) Nuclei (2×10^8) were incubated with 25 μCi of [γ-^{32}P]ATP for 10 min at 37 °C and processed as described in (A). (C) Nuclei (2×10^8) were incubated with 25 μCi of [γ-^{32}P]ATP and extracted for histone H1 as described in (A). Phosphorylated H1 was treated with 0.2 unit of phosphatase-free snake venom phosphodiesterase (Worthington) for 10 min at 25 °C prior to loading onto the column.

neous phosphorylation and poly(ADP-ribosylation) of histones. HeLa nuclei were incubated with [^{3}H]NAD and/or [γ-^{32}P]ATP, and the amount of radioactivity incorporated into the acid-insoluble fraction was determined (Table I). It was observed in this experiment that both the cAMP-dependent (Langan, 1971) and -independent nuclear kinase(s) (Gurley et al., 1974; Balhorn et al., 1975) could be utilized in combination with nuclear poly(ADP-Rib) polymerase to radioactively label nuclear components for subsequent extraction of histone H1. It was additionally noted that the presence of NAD slightly reduced the nuclear kinase activity and cAMP lowered ADP-ribosylation to a limited extent.

Binding of Phosphorylated H1 to Anti-Poly(ADP-Rib)–Sepharose. Nuclei were incubated with [γ-^{32}P]ATP in the presence (Figure 4A) or absence (Figure 4B) of cAMP and selectively extracted with 5% perchloric acid for histone H1. Electrophoretic and autoradiographic analyses indicated various ^{32}P-labeled bands of histone H1. In the absence of cAMP, a few contaminating bands were also evident (data not shown). The samples were subsequently subjected to chro-

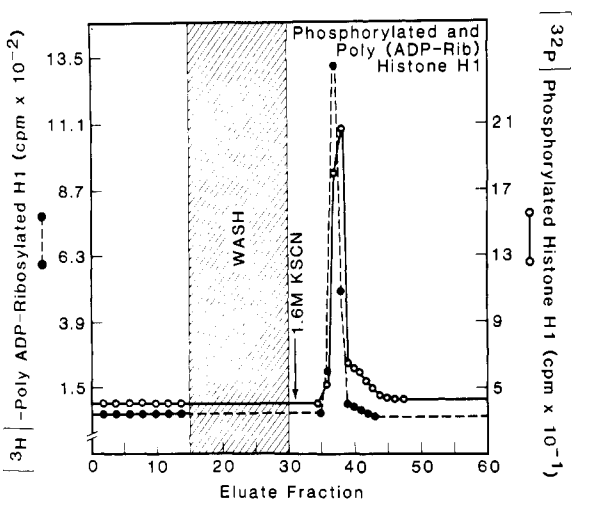

FIGURE 5: Association of phosphorylated H1 with poly(ADP-ribosylated) H1 as determined by anti-poly(ADP-Rib) IgG immunofractionation. Nuclei (3×10^8) were incubated with 30 μCi of [γ-^{32}P]ATP and 12 μCi of [^{3}H]NAD for 10 min at 37 °C as described under Materials and Methods. Histone H1 was selectively extracted and analyzed as described in Figure 2. (○) ^{32}P-phosphorylated H1; (●) ^{3}H-labeled poly(ADP-ribosylated) H1.

matography on anti-poly(ADP-Rib)–Sepharose as described earlier. Unexpectedly, greater than half of the phosphorylated H1 sample was observed to bind to the poly(ADP-Rib) antibody column (Figure 4A,B). Several possibilities could account for these observations: (i) The results might be due to the nonspecific binding of phosphorylated protein to the column. (ii) The in vitro conversion of the γ-phosphate of ATP into NAD may be occurring in washed nuclei; however, this would be highly unlikely based upon past experiments (Uhr & Smulson, 1982). Furthermore, "tracer" quantities of labeled NAD would yield only mono(ADP-ribosylated) H1, which is not retained by the poly(ADP-Rib) antibody column (Figure 2). (iii) Endogenous poly(ADP-ribosylated) H1 species (i.e., modified in vivo, prior to nuclear isolation) may be particularly accessible to phosphorylation. The endogenous pools of such poly(ADP-ribosylated) molecules would be expected to be quite small (Stone et al., 1977); however, their presence has been recently demonstrated (Wong et al., 1983). The latter possibility was tested in the experiments shown in Figure 4C.

Phosphorylated histone H1 was treated with snake venom phosphodiesterase which cleaves either free poly(ADP-Rib) or polymer which is covalently bound to acceptors (Butt et al., 1980). The sample contained 3150 acid-insoluble cpm prior to treatment and 3100 cpm after the treatment. Accordingly, little if any ^{32}P-labeled poly(ADP-Rib) was present in the sample. Subsequently, the phosphodiesterase-treated [^{32}P]-ATP-labeled sample was rechromatographed on the antibody column (Figure 4C). In contrast to the earlier data, essentially all of the phosphorylated H1 sample eluted directly through the column. These experiments suggested that a large percentage of HeLa cell nuclear histone H1 occupies domains in chromatin accessible to both phosphorylation and poly(ADP-ribosylation).

To further study the relationship between the two post-translational modifications of H1, nuclei were double labeled with 50 μM [^{3}H]NAD and 18 μM [γ-^{32}P]ATP. Whereas endogenous levels of poly(ADP-ribosylated) H1 are extremely low in isolated nuclei (Wong et al., 1983), poly(ADP-ribosylation) in vitro with 50 μM NAD causes extensive polymer synthesis (Nolan et al., 1980), which is sufficient for maximal retention of chromatin to the antibody column (Malik et al., 1983). In the experiment shown in Figure 5, it was noted

that the phosphorylated species of histone H1 showed total affinity for the poly(ADP-Rib) antibody and coeluted with the poly(ADP-ribosylated) molecules.

Discussion

We have recently prepared an antibody preparation specifically directed against the posttranslational moiety, poly(ADP-Rib), to investigate a number of hitherto unattainable parameters concerning poly(ADP-ribosylation). For example, by use of immunofluorescence staining techniques, the content of poly(ADP-Rib) in cells has been determined during the cell cycle (Kanai et al., 1981). Maximal poly(ADP-Rib) synthesis was observed in G2-M phases. Also, the antibody was used to demonstrate the natural occurrence of poly(ADP-ribosylated) species of H1 and the cross-linked, H1 complex, in vivo (Wong et al., 1983). Perchloric acid extracts from synchronously growing HeLa cells were directly fractionated by electrophoresis, transferred to nitrocellulose, and probed with the antibody. More recently, antibody coupled to Sepharose was utilized to isolate selectively from the bulk of chromatin oligonucleosomal domains (10% of total) undergoing the poly(ADP-ribosylation) reaction (Malik et al., 1983). The data indicated that the poly(ADP-ribosylated) oligonucleosomal DNA contained significant amounts of internal single-strand breaks compared with bulk chromatin. This observation might be related to the proposed biological role for this system in DNA repair/replication (Thraves & Smulson, 1982; Sudhakar et al., 1979).

In the current study the use of the anti-poly(ADP-Rib) has been extended to isolate specifically the poly(ADP-ribosylated) species of histone H1 and to study its relationship to H1 phosphorylation.

Chromatin undergoes transient alterations in structure during the cell cycle. Certain levels of the phosphorylation of histone H1 are related to cAMP-dependent activation of gene expression (Langan, 1971; Takeda & Ohga, 1973; Wicks et al., 1975). Alternatively, during S phase, chromatin must be made accessible to DNA polymerase. Later in the cell cycle (i.e., G2–mitosis), H1 undergoes phosphorylation at several sites in relation to the mechanism of chromatin compaction and the formation of higher ordered structures (Bradbury et al., 1973; Gurley et al., 1978; Sealy & Chalkley, 1978; Halleck & Gurley, 1980; Matsumoto et al., 1980). It is of significance that poly(ADP-ribosylation) of H1 seems to be especially prominent in these same latter phases of the cell cycle (G2–mitosis) and also appears to play a role in polynucleosome condensation (Wong et al., 1982, 1983). For this latter role, the poly(ADP-ribosylation) mechanism is particularly suitable. A cross-linked dimer of H1 connected by a 15–16-unit poly(ADP-Rib) chain is a prominent product of this reaction. The data in Figure 3 indicated that this product could be selectively purified by the anti-poly(ADP-Rib) immunoaffinity procedure. By utilizing the same antibody, we recently showed that the H1 dimer was maximally synthesized in intact cells at the S-G2 phase boundary of the cell cycle (Wong et al., 1983). The H1 complex, like phosphorylated H1, may be a transient modification of this histone, which causes localized condensation of chromatin after periods of relaxation occurring during transcription, replication, or repair. We have hypothesized that the formation of this H1 cross-link may help stabilize nucleosomal domains containing DNA strand breaks during DNA replication and repair (Wong et al., 1982). The levels of polymer and the poly(ADP-ribosylation) of H1 are significantly increased in cells exposed to agents which cause DNA strand breaks (Thraves & Smulson, 1982; Sudhakar et al., 1979; Berger et al., 1979). It is worth noting that the level of nuclear protein phosphorylation has been reported to be elevated following the in vivo exposure of Yoshida ascites tumor cells sensitive to alkylating agents (Riches et al., 1977).

As with histone H1 phosphorylation in the G2-M phase, poly(ADP-ribosylation) of the same proteins may also function to condense chromatin. Purified oligonucleosomes have been shown (by electrophoretic and sedimentation velocity studies) to condense in vitro in a NAD concentration dependent fashion, and with the generation of extensive polymer chain lengths (Nolan et al., 1980; Butt & Smulson, 1980). Furthermore, this condensation process was observed to be lost upon selective removal of histone H1 from chromatin (Butt et al., 1980). More recently this effect was restored upon the reconstitution of H1 with H1-depleted nucleosomes, under conditions of "native" poly(ADP-ribosylation) levels of this histone.

The question arises as to whether phosphorylation and poly(ADP-ribosylation) of histone H1 might mutually participate at some level of higher ordered chromatin condensation. The data presented above (Figures 3–5) suggest that certain species of histone H1 coordinately become both phosphorylated and poly(ADP-ribosylated) in vitro. These results are somewhat analogous to the observation of Whitlock et al. (1980), who have found that acetylated forms of histone H3 are particularly susceptible to phosphorylation.

A phosphorylated sample of histone H1 was observed to possess significant binding to anti-poly(ADP-Rib) IgG. This was presumedly due to the presence of endogenous poly(ADP-ribosylated) H1 species, since treatment of the ^{32}P-phosphorylated H1 sample with phosphodiesterase [i.e., removing poly(ADP-Rib)] eliminated binding of the sample to the antibody column (Figure 4C).

Poly(ADP-ribosylation) sites on histone H1 (from rat liver) have been established to be glutamic acid residues at 2 and 14 from the NH_2-terminal and the COOH-terminal lysine (Ogata et al., 1980). In contrast, site specificity of phosphorylation of H1 species is complicated by the cell cycle related regulations (Hohmann et al., 1976). In the G1 phase, most H1A species contain zero or one phosphate group and most H1B molecules contain zero to three phosphates. Both H1 molecules undergo a general increase in the phosphorylation levels of about one phosphate per mole during the S phase and a further increase during mitosis (Ajiro et al., 1981a,b).

The phosphorylation sites on H1 appear to be predominately serine and threonine residues, thus allowing for the possibility of mutual poly(ADP-ribosylation) and phosphorylation. We have no information, at this time, on the sites of in vitro phosphorylation or which species and/or amino acid residues of H1 are retained by the poly(ADP-Rib) antibody column, although it has recently been reported that in vitro phosphorylation is quite similar to the in vivo residue modification (Sun & Allfrey, 1982).

One possible interpretation derived from Figure 4 that more than half of the H1 molecules which are phosphorylated in vitro are those which have been poly(ADP-ribosylated) in vivo should be tempered at this stage in the analysis. In growing cells, a significant fraction of total H1 molecules is phosphorylated, yet the fraction of H1 molecules which are poly(ADP-ribosylated) is extremely small. There is a strong possibility that phosphorylation with isolated nuclei is also restricted to small domains of H1-containing chromatin, accessible to labeled ATP and endogenous kinases, under these in vitro conditions. The data imply that these extended regions of chromatin are experimentally accessible to both histone H1

phosphorylation and poly(ADP-ribosylation), in a manner similar to that noted by Whitlock et al. (1980) for endogenous acetylation and in vitro phosphorylation of histones H3 and H4. Clearly, in vivo correlative experiments need to be performed relating the two H1 modification systems. However, the development of the present immunoaffinity methodology to study modified moieties on nuclear proteins should allow many of these questions to be answered by future approaches.

Registry No. Poly(ADP-Rib), 26656-46-2.

References

Ajiro, K., Borun, T. W., & Cohen, L. (1981a) *Biochemistry 20*, 1445–1454.

Ajiro, K., Borun, T. W., Shulman, S. D., McFadden, G. M., & Cohen, L. H. (1981b) *Biochemistry 20*, 1454–1464.

Balhorn, R., Jackson, V., Granner, D., & Chalkley, R. (1975) *Biochemistry 14*, 2504–2511.

Berger, N., Petzold, S. J., & Berger, S. J. (1979) *Biochim. Biophys. Acta 546*, 90–104.

Bradbury, E. M., Inglis, R. J., Mathews, H. R., & Sarner, N. (1973) *Eur. J. Biochem. 33*, 131–139.

Butt, T. R., & Smulson, M. (1980) *Biochemistry 19*, 5235–5242.

Butt, T. R., Decoste, B., Jump, D. B., Nolan, N., & Smulson, M. (1980) *Biochemistry 19*, 5243–5249.

Gurley, L. R., Walters, R. A., & Tobey, R. A. (1974) *J. Cell Biol. 60*, 356–364.

Gurley, L. R., Walters, R. A., Barham, S. S., & Deaven, L. L. (1978) *Exp. Cell Res. 111*, 373–383.

Halleck, M. S., & Gurley, L. R. (1980) *Exp. Cell Res. 125*, 377–388.

Hohmann, P., Tobey, R. A., & Gurley, L. R. (1976) *J. Biol. Chem. 251*, 3685–3692.

Kanai, Y., Miwa, M., Matsushima, T., & Sugimura, T. (1974) *Biochem. Biophys. Res. Commun. 59*, 300–306.

Kanai, Y., Tanuma, S., & Sugimura, T. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 2801–2804.

Kidwell, W. R., & Mage, M. G. (1976) *Biochemistry 15*, 1213–1217.

Langan, T. A. (1971) *J. Biol. Chem. 244*, 5763–5765.

Malik, N., Miwa, M., Sugimura, T., & Smulson, M. (1983) *Proc. Natl. Acad. Sci. U.S.A.* (in press).

Matsumoto, Y., Yasuda, H., Mita, S., Marumonchi, T., & Yamada, M. (1980) *Nature (London) 284*, 181–183.

Mullins, D. W., Giri, C. P., & Smulson, M. (1977) *Biochemistry 16*, 506–513.

Nolan, N., Butt, T., Wong, M., Lambrianidou, A., & Smulson, M. (1980) *Eur. J. Biochem. 113*, 15–25.

Ogata, N., Ueda, K., Kagamiyama, H., & Hayaishi, O. (1980) *J. Biol. Chem. 255*, 7616–7620.

Riches, P. G., Gellwood, S. M., & Harrap, K. R. (1977) *Chem.-Biol. Interact. 18*, 11.

Rickwood, D., Riches, P. G., & MacGillivray, J. (1973) *Biochim. Biophys. Acta 299*, 162–171.

Sealy, L., & Chalkley, R. (1978) *Cell (Cambridge, Mass.) 14*, 115–121.

Sporn, M. B., Berkowitz, D. M., Glinki, R. P., Ash, A. B., & Steven, C. L. (1969) *Science (Washington, D.C.) 164*, 1408–1410.

Stone, P. R., Lorimer, W. S., & Kidwell, W. R. (1977) *Eur. J. Biochem. 81*, 9–18.

Sudhakar, S., Tew, K., Schein, P., Wooley, P., & Smulson, M. (1979) *Cancer Res. 39*, 1411–1417.

Sun, I. Y. C., & Alfrey, V. G. (1982) *J. Biol. Chem. 257*, 1347–1353.

Takeda, M., & Ohga, Y. (1973) *J. Biochem. (Tokyo) 73*, 621–629.

Tanuma, S., & Kanai, Y. (1982) *J. Biol. Chem. 257*, 6565–6570.

Thoma, F., & Koller, T. H. (1977) *Cell (Cambridge, Mass.) 12*, 101–107.

Thraves, P. J., & Smulson, M. (1982) *Carcinogenesis (London) 3*, 1143–1148.

Uhr, M. L., & Smulson, M. (1982) *Eur. J. Biochem. 128*, 435–443.

Whitlock, J. P., Jr., Augustine, R., & Schulman, H. (1980) *Nature (London) 287*, 74–76.

Wicks, W. D., Koontz, J., & Wagner, K. (1975) *J. Cyclic Nucleotide Res. 1*, 49–58.

Worcel, A. (1978) *Cold Spring Harbor Symp. Quant. Biol. 42*, 313–324.

Wong, M., Malik, N., & Smulson, M. (1982) *Eur. J. Biochem. 128*, 209–213.

Wong, M., Kanai, Y., Miwa, M., Bustin, M., & Smulson, M. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 205–209.