Monoclonal Antibodies Specific for Tight-binding Human Chromatin Antigens Reveal Structural Rearrangements within the Nucleus during the Cell Cycle

JASWANT S. BHORJEE, STEPHEN L. BARCLAY, ANDRZEJ WEDRYCHOWSKI, and ALAN M. SMITH
Department of Biological Sciences, University of Illinois-Chicago, Chicago, Illinois 60680

ABSTRACT The class of nonhistone chromosomal proteins that remains bound to DNA in chromatin in the presence of 2.5 M NaCl-5 M urea has proven refractile to biochemical analysis. In order to study its role in chromatin organization, we have produced monoclonal antibodies that are specific for the HeLa DNA-protein complex that remains after extraction of chromatin with high salt and urea. The antibody-producing clones were identified with an ELISA assay. Of the six clones selected, five were stabilized by limiting dilution. All clones are IgG producers. None cross-react significantly with native DNA, core histones, or the high-mobility group nonhistone proteins. All antibodies are specific for nuclear or juxtanuclear antigens. Indirect immunofluorescence shows that three antibodies, which are nonidentical, stain three different nuclear networks. Available evidence indicates that two of these networks are the nuclear matrix. A fourth antibody reveals structures reminiscent of chromocenters. A fifth antibody, AhNA-I, binds to interphase HeLa chromatin and specifically decorates metaphase chromosomes. AhNA-I similarly recognizes rat chromosomes. Each of these monoclonal antibodies also reveals a changing pattern of nuclear staining as cells progress through the cell cycle. Presumably, this reflects the rearrangement of the cognate antigens.

The eucaryotic chromosome contains at least twice as much protein by mass as DNA and both are complexed in a structure defined as chromatin. Treatment of either purified nuclei or chromatin by high salt (2.0 M NaCl) removes almost all histones and most nonhistone proteins (1-3). The proteins remaining bound to DNA in chromatin after high ionic strength extraction (2 M NaCl or 2 M NaCl-5 M urea) are nonhistones, and they constitute 5-8% of the total chromatin protein. These are termed residual or tight-binding proteins (4-6). The tight-binding, nonhistone chromatin proteins (TBP) from animal cell nuclei are electrophoretically complex (~200 species, Bhorjee, J. S. and L. Kife, unpublished data), and are distributed nonrandomly along the DNA molecule (reference 5, reviewed in reference 6).

Two functions for these proteins have been suggested, although no direct evidence exists. Based on the DNA sequence-specific association of certain nonhistone proteins with chromatin DNA, a role in specific gene expression for these proteins has been proposed (7-13). It has also been demonstrated that some TBPs bind to androgen-receptor (14) and progesterone-receptor (15) complexes. Thus, at least some gene regulatory molecules in eukaryotes may reside in this group of chromosomal proteins. A second function may be structural. The structural integrity of the nuclear matrix (4, 16) and the chromosome scaffold (17) seems to rely on the presence of such high-ionic strength (2 M NaCl), nonextractable nuclear nonhistone proteins.

Closer examination of these proposals by biochemical analysis of individual tight-binding nonhistone proteins has not been possible for two reasons: (a) each is present in small amounts and (b) dissociation of the DNA-protein complex requires denaturing solvents that may lead to irreversible loss of functions. Therefore, to avoid these problems and to study the structure and function of HeLa tight binding chromatin proteins, we have prepared monoclonal antibodies against this group of nonhistone proteins. We present here an initial characterization of several HeLa TBP-specific monoclonal antibodies and their antigens. In addition, we use indirect
immunofluorescence to demonstrate that the spatial distributions of these antigens change dramatically during the cell cycle.

MATERIALS AND METHODS

Cell Growth, Chromatin Isolation, and TBP Fractionation:
HeLa cells were grown in suspension cultures in Joklik-modified Eagle's medium (18), and chromatin was purified from isolated nuclei by sedimentation through 60% sucrose as previously described (19). TBPs were fractionated from purified chromatin by treatment with 2.5 M NaCl-5 M urea followed by sedimentation of the TBP-nucleoprotein complex at 408,000 g for 24 h as described (5). This TBP-nucleoprotein complex was used as an immunogen. All steps were performed at 4°C and all solutions contained 0.1 mM PMSF as a protease inhibitor. Where indicated in the text, samples were divided by the double-thymidine block procedure as described previously (20).

Immunological Detection of Proteins by Electrophoretic Blotting:
The TBPs were dissociated from the TBP-nucleoprotein complex, as described above, by treatment with 2% sodium dodecyl sulfate (SDS), 1% 2-mercaptoethanol (BME), at room temperature for 30 min with occasional vortexing. The sample was then dialyzed overnight against Tris-NaCl buffer (0.01 M NaCl, 0.002 M Tris-HCL pH 7.2) containing 1% SDS-1% BME and centrifuged at 150,000 g for 20 h in Beckman SW50.1 rotor (Beckman Instruments, Inc., Spinc Div., Palo Alto, CA) at 20°C, to pellet DNA. Almost all of the protein (>99%) was in the supernatant and DNA (>98%) was in the pellet, as determined by [3H]thymidine and [3H]leucine labeling, respectively. The cytoplasmic proteins were obtained by centrifuging the postnuclear supernatant (19) at 3,000 g for 15 min in Sorval SS-34 rotor at 4°C. The nuclear matrix proteins were obtained by the procedure of Berezney and Coffey (16), except for the pretreatment of isolated nuclei with DNase I (100 µg/ml DNA) for 30 min at 22°C to avoid gel formation in the high-salt buffer treatment step. Various protein samples (TBPs; nuclear matrix) were treated with 1% SDS-1% BME, heated at 90°C for 2 min, and dialyzed against Laemmli sample buffer (21) before electrophoresis.

The electrophoretic transfer and immunological detection of the electrophoretic blots were essentially as described by Towbin et al. (22). Briefly, the proteins were first separated in a 1% polyacrylamide SDS-slab gel and transfered electrophoretically to a nitrocellulose membrane sheet using Hoeffer "Transblot" apparatus. The electrophoretic blot membrane strips were incubated with a twofold dilution of the monoclonal supernatant, washed, and further incubated with 125-I-labeled goat anti-mouse IgG (Amersham Corp., Arlington Heights, IL). The strips were then washed free of the unreacted secondary antibody and exposed to Kodak X-Omat AR film in the presence of an intensifying screen.

Plasmacytoma Culture Conditions:
The nonsecreting, 8-aza-guanine-resistant mouse plasmacytoma line SP2/0-Ag14 was grown in high glucose Dulbecco's modified Eagle's medium (DME) supplemented with 20% gamma horse serum (KC Biologicals), penicillin, and streptomycin. Cultures were maintained between 10^3 and 7 x 10^5 cells/ml at 37°C in a humid chamber containing 5% CO2.

Immunization and Preparation of Hybridomas:
BALB/c mice (12-16 wk old) were immunized by intraperitoneal injection of 0.5 ml of immunogen in PBS (100 µg/ml). Animals were boosted at 3-wk intervals by i.p. injection as above. 60-72 h after the sixth injection (into the tail vein), dissociated spleen cells were prepared from an immunized animal and were fused with SP2/0 cells using 5% polyethylene glycol (PEG 1500, Fisher Scientific Co., Pittsburgh, PA) as described by McKearn et al. (23). Treated cells were incubated overnight at 37°C in 5% CO2 in high glucose DME containing 20% gamma horse serum. Cells were harvested the following day and were resuspended in high glucose DME containing 20% gamma horse serum, 1 x 10^7 M hypoxantine, 4 x 10^-7 M aminopterin, 3 x 10^-4 M thymidine, L-arginine (0.116 g/l), L-glutamine (0.216 g/l), folic acid (0.006 g/l), L-asparagine (0.036 g/l), sodium bicarbonate (2.0 g/l), and sodium pyruvate (0.11 g/l). Cells were distributed in 100-µl aliquots into 400 or more microtiter wells and incubated at 37°C in 5% CO2, 5-6 d later, hybridomas were fed with an additional 100 µl of the above-described medium lacking hypoxantine, aminopterin, and thymidine. A similar protocol using Freud's adjuvant gave a similar frequency of positive hybridomas.

Screening of Hybridomas and Preparation of Monoclonal Antibodies:
Positive hybridomas were detected by an ELISA assay (24). The high salt-urea insoluble DNA-protein complex prepared as described above was used as the immunogen. Where indicated in the text, samples were divided by the double-thymidine block procedure as described previously (20).

RESULTS

Preparation of Immunogen and Generation of Hybridoma Clones

The DNA-protein complex that remains after extraction of HeLa chromatin with 2.5 M NaCl-5 M urea was used as an immunogen to raise monoclonal antibodies. Some physical and chemical properties of this group of high-affinity DNA-binding nonhistone proteins have been previously described. For example, the TBPs constitute 5-8% of the total chromatin protein, are nonhistones, are not related to the proteins of the heterogeneous ribonucleoprotein particles, and are distributed asymmetrically along the DNA molecule (5).

This nucleoprotein complex is a weak immunogen. In two separate fusions, not more than 2% of the hybridomas secreted antibodies that reacted with the respective antigens as determined by the ELISA assay (24). At least five injections were necessary before a weak positive antibody response could be detected. We have identified six positive clones, and five of these have been stabilized by several subclonings, thus assuring the monoclonal nature of each of the stabilized clones.

Characterization of the Monoclonal Antibodies

We examined five stabilized hybridoma lines for their antigen specificity by using the ELISA assay (see Materials and Methods). All five hybridomas are IgG producers. Table I shows that the antigen-antibody binding reaction is specific for the 2.5 M NaCl-5 M urea stable nucleoprotein complex (TBP). We have numbered the antibody-producing clones with the prefix AhNA for anti-human nuclear antigen. Clones AhNA-4 and AhNA-5 give a strong ELISA reaction and AhNA-2 gives the weakest response. All antibodies are reactive with the native complex of HeLa TBP (column 3). This reactivity remains even after digestion of the native complex with DNase I (column 9). HeLa double-stranded DNA does not react with these antibodies. The weak reactions of HeLa single-stranded DNA and HeLa HMGs occur only when these components are present in the assay in amounts that are far greater than are found in the native complex. The weak reaction of some antibodies with preparations of HeLa cytoplasm is expected to result from dispersal of some nuclear antigens into the cytoplasm arising from a minimum number
### TABLE I
ELISA Assay of Subcellular Fractions

| Antigen source | HeLa TBP (native complex) | HeLa dsDNA | HeLa ssDNA | HeLa HMGs | HeLa CP | HeLa NM | DNase I sensitivity of the TBP complex* |
|----------------|---------------------------|------------|------------|-----------|--------|--------|----------------------------------------|
| AhNA1          | IgG                       | 3+         | -          | ±         | -      | -      | -                                      |
| AhNA2          | IgG                       | 3+         | -          | -         | ±      | -      | -                                      |
| AhNA3          | IgG                       | 3+         | ±          | -         | -      | -      | -                                      |
| AhNA4          | IgG                       | 4+         | ±          | -         | -      | -      | ± 2+                                   |
| AhNA5          | IgG                       | 4+         | ±          | ±         | ±      | ±      | ± 2+                                   |
| AhNA6          | NT                        | 3+         | NT         | NT        | NT     | NT     | NT                                     |

* Determined by Ouchterlony assay.

+, Indicates the rate of reaction; for example, 4+ wells positive in 20 min; 3+ in 45 min; 2+ in 90 min. The amount of antigen per well within a column was kept constant. Also, in assays for TBP, HMGs, CP, and NM—0.75 μg of total protein was added per well.

±, Slightly above background.

NT, Not tested.

### TABLE II
Species Specificity and the Cellular Distribution of the HeLa DNA-TBP Chromatin Antigen as Determined by Indirect Immunofluorescence

| Clone | Cellular localization | Cell type | Distribution Pattern | Pattern frequency* |
|-------|-----------------------|-----------|----------------------|--------------------|
| AhNA1 | Nuclear               | HeLa      | Metaphase chromosomes; punctate in interphase cells | >90% punctate (Fig. 1, bottom right); 5–10% chromosomes |
| AhNA2 | Nuclear               | HeLa      | Ranges from one large spot or crescent-shaped to granular (chromocenter) | 60–65% crescent-shaped; 20–30% large spot; 5% weak to no reaction |
| AhNA3 | Nuclear               | Rat muscle| Matrixlike pattern   | >70% matrixlike; remainder threadlike to weak reaction |
| AhNA4 | Nuclear               | HeLa      | Matrixlike pattern   | 80% matrixlike; remainder weak to no reaction |
| AhNA5 | Nuclear               | HeLa      | Mostly matrix pattern| 70–75% matrixlike (similar to AhNA3) |
| AhNA6 | Nuclear               | NT        | Patchy              | 80% patchy |

NT, not tested.
±, faint fluorescence.

* In all cases a minimum of 200 cells were observed.

of mitotic cells in the unsynchronously growing population or synthesis of the antigen in the cytoplasm. The reaction of two of these antibodies with HeLa nuclear matrix proteins is analyzed later in this paper.

**Cellular Localization and Distribution Pattern of the Antigens by Indirect Immunofluorescence**

Table II lists the immunofluorescent staining properties of antibodies produced by the five stabilized clones and by one clone (AhNA-6) that has not been stabilized. Two mammalian cell lines (HeLa and L8 rat skeletal muscle) were tested by immunofluorescence. Fig. 1 shows the immunofluorescent staining patterns summarized by Table II. The frequencies of the immunofluorescent patterns for various antibodies are also given in Table II. Clearly, the interphase cells display the dominant staining patterns seen in Fig. 1.

Comparative phase contrast (not shown) and immunofluorescence micrographs (Fig. 1) indicate that the cognate antigens of these antibodies are arranged in distinctive patterns within the nucleus. Although some antibodies revealed staining juxtaposed to the nuclear membrane (e.g., AhNA 2, Fig. 1), as far as could be discerned, no cytoplasmic fluorescence was evident. Nor is the nuclear staining so diffuse as found for heterogeneous nuclear RNA-ribonucleoprotein antigens (26). The staining patterns are always highly reproducible within and between experiments.

The most readily interpretable staining is the specific decoration of metaphase chromosomes by AhNA-1 (Fig. 1). This antigen is present in two cell types and species (HeLa cells and rat skeletal myoblasts) since both cell types show identical patterns of staining of metaphase chromosomes (rat not shown). No other monoclonal antibodies tested stain mitotic chromosomes in this pattern (see Fig. 3).
FIGURE 1 Indirect immunofluorescence photomicrographs of unsynchronized HeLa cells treated with mouse monoclonal antibodies to the HeLa TBPs. Bars, 10 μm.
and 4 (Fig. 1). Thus, two different matrixlike patterns are
always more delicate than that made apparent by AhNA-3
antigens in ELISA (Table I) and "Western" blots (Fig. 2) raises
the possibility that the observed staining in Fig. 1 corresponds
to the nuclear matrix. Monoclonal antibody AhNA-5 is
clearly different form AhNA-3 and 4. It does not form an
immunocomplex with either TBP or nuclear matrix proteins
on "Western" blots or TBP solubilized by any of our current
methods. Thus, we have not determined the molecular weight
of its antigen. The matrixlike pattern that AhNA-5 shows is
always more delicate than that made apparent by AhNA-3
and 4 (Fig. 1). Thus, two different matrixlike patterns are
made visible by these antibodies.

The unusual immunofluorescent configurations are not
artifacts of the formaldehyde cell fixation procedure, as shown
by the following. First, an alternate fixation procedure using
acetone gives identical immunofluorescent patterns. Second,
the AhNA-1 specific staining of metaphase chromosomes is
undisturbed by fixation, thereby constituting a strong control
for chromatin staining. Furthermore, the absence of staining
by secondary antibody alone also indicates that these mono-
clonal antibodies are specific for HeLa chromatin nonhistone
proteins.

Finally, AhNA-6 reveals a "patchy" nuclear distribution of
its antigen. This pattern may be that of a heterochromatin-
specific polypeptide. Its immunofluorescence pattern is defi-
nitely not like the matrixlike pattern given by AhNA-3, 4, or
5. Although many cells have been examined by indirect
staining with AhNA-6, none have threadlike, criss-crossing
structures shown by AhNA-3, 4, or 5. This antibody has not
been extensively studied because the hybridoma line has not
yet been stabilized.

Cell Cycle and Nuclear Immunofluorescence

Physical and chemical alterations in chromatin structure
are known to occur at different stages of the cell cycle in HeLa
(20, 28, 29). Cytological evidence, using the fluorochrome
quinacrine, suggests a continuous chromosome cycle through-
out interphase in human (30) and mouse (31) cells. In order
to explore the relationship between chromatin organization
and the cell cycle, we have used three of the monoclonal
antibodies to determine whether the structural organization
of their antigens within the nucleus changes as HeLa cells
progress through the cell cycle.

Fig. 3 illustrates that the organization of the antigens of
AhNA-1, 2, and 3 changes dramatically as cells progress
through the cell cycle. Although these antibodies reveal dis-
tinctly different patterns, one common feature observed is the
fluorescent patterns that cover the entire cell during mitosis,
for example, panels for AhNA-2 and 3 in Fig. 3. Because the
nuclear envelope breaks down at metaphase, one should not
be surprised to find that nucleoplasmic proteins are released
into the cytoplasm at this stage. The diffuse staining is not an
artifact because AhNA-1 shows localized fluorescence at mi-
rosis. We interpret the staining pattern of AhNA-2 and 3 at
mitosis in the Discussion. In the following, we note particular
features revealed by AhNA-1, 2, and 3.

Fig. 3 (top panel) shows the immunofluorescent patterns
obtained with AhNA-1. Cells in G1 have a distinctly granular
pattern throughout the nucleus. At mitosis, fluorescence is
highly localized in patterns that suggest condensed chromo-
somes which reverses to a granular pattern again in G2 (see
also Fig. 1). We interpret these results to mean that the antigen
of AhNA-1 is bound to both interphase chromatin and con-
densed chromosomes.

Monoclonal antibody AhNA-2 recognizes a tight-binding
protein which is regionalized in the nucleus. G1 stage cells
show a single, circular structure which often has the appear-
ance of a stack of carefully coiled threads (Fig. 3, middle
panel). In some instances, these threads radiate outwardly to
lie beneath the nuclear envelope. At mitosis, the pattern is
granular, covering the entire cell, and in G2 cells, one sees
the return of the G1-like bright fluorescent center within the
nucleus. This staining pattern is highly reproducible. Between
60 and 80% of cells in the synchronized population show the
characteristic bright spotlike fluorescence of G1 and G2 cells.

Although we cannot yet confirm this inference, the immu-
nofluorescence pattern of AhNA-2 suggests that its antigen is
a part of heterochromatin and that the dense bright, coiled
Figure 3 Changing patterns of nuclear distribution of the cognate antigens as revealed by indirect immunofluorescence of synchronized HeLa cells treated with monoclonal antibodies: AhNA-1 (top panel), AhNA-2 (middle panel), AhNA-3 (bottom panel). Synchronized cells were harvested at 5 h (G1), 8 h (G2), 10 h (M), and 13 h (early G1) after release from the thymidine block (20). Note: the micrograph for AhNA2-G1 has been spliced to juxtapose two cells in the same view.

Structure is a "chromocenter," as defined by Natarajan and Gropp (32). We are unaware of any immunofluorescence studies that have directly visualized a chromocenter of human cells. However, earlier cytological studies using Feulgen staining and fluorochrome dyes suggested that chromocenters are formed by the fusion of constitutive heterochromatin in the mouse (32, 33). A disperse, faintly quinacrine fluorescent pattern in late G1 to bright fluorescent granular pattern in late G2 and prophase of constitutive heterochromatin (=chromocenters) in HeLa and mouse fibroblast nuclei has been shown to occur as cells progress through the cell cycle (30, 31). Furthermore, the number and size of chromocenters seem to vary with the cell cycle stage, being an average of 13 chromocenters per nucleus in late G1 to 35 chromocenters per nucleus in late G2 to prophase in the mouse (31). The sequence of the bright spotlike immunofluorescence in G2 to granular pattern in M and return to dense bright structure in G1 for the AhNA-2 antigen (Fig. 3, middle panel) suggests possible alterations in the functional states of constitutive heterochromatin, i.e., the chromocenter, if, indeed, this chromatin arrangement can be equated with the quinacrine staining appearance of chromocenters (30–32).

Fig. 3 (bottom panel) illustrates the staining pattern of AhNA-3. In both G1 and G2 cells a filamentous, matrixlike pattern is seen in the nucleus (see also Fig. 1). This pattern is reproducible and is displayed by 70% or more cells within an unsynchronized population (Table II). Staining of the cytoplasm does not occur in G1 or G2 cells. In contrast, cells in mitosis show a diffuse, but slightly granular, pattern of fluorescence that includes the entire cell. As noted above and in
the cytoplasm when the nuclear envelope breaks down.

We note here as a word of caution that S-phase cells obtained by the double-thymidine block synchronization procedure displayed an abnormal diffuse fluorescence over the total cell, regardless of the antibody used. This may be due to the known cytotoxic effects of the high thymidine concentrations used for induction of cell synchrony, which have been shown to cause reversible unbalanced growth, abnormal cell metabolism, and nuclear damage (34–37).

**DISCUSSION**

Only a few studies have used monoclonal antibodies as reagents to identify and survey the spatial distribution of chromosomal proteins (38, 39, 41, 42, 44). Our study differs from the previous ones in two ways. First, we have focussed specifically on chromatin-associated proteins that remain bound in a DNA-protein complex in the presence of 2.5 M NaCl-5 M urea. The significance of these proteins was discussed in the Introduction. Second, we have examined the subcellular localization of these antigens throughout the cell cycle.

The antigen identified by AhNA-1 is clearly associated with chromatin. The entire metaphase chromosome is visualized by immunofluorescent staining with AhNA-1. This is in contrast to regional staining, i.e. staining of only the termini, as observed by Will and Bautz (27) who used a polyclonal antisera specific for heterochromatin. The cognate antigen of AhNA-1 is organized in the chromosome in a way that makes it readily accessible to the antibody. This antigen is unlike the one identified by Turner (38), which becomes accessible to the antibody only after salt washing of nuclear preparations. AhNA-1 does not cross-react with HeLa core histones. Therefore, the even distribution of fluorescence along the metaphase chromosome does not result from reaction of the antibody with core histones. The granular nuclear staining in other cell cycle stages suggests that this antigen is present in chromatin throughout the cell cycle. We have shown that the antigen is not DNA, core histones, or HMG proteins (Table I). Thus, it appears that a new component of chromatin has been identified. This antigen is also present in rat chromatin.

Other workers have shown that antigens present in total nonhistone protein preparations are widely distributed along the entire length of individual polytene chromosomes (40). In one case, a cognate antigen of a monoclonal antibody that is specific for a nonhistone protein is known to be localized in regions of active or potentially active genes (41). The antigen (AhNA-1) we identify may similarly be localized in active (or inactive) gene regions, but we cannot test this directly by immunofluorescence methods because of cytological limitations inherent to nonpolytene chromosomes. Another alternative that we hope to test is that this antigen is part of the chromosome scaffold (17).

The pattern of immunofluorescence given by AhNA-2 and AhNA-3 is apparently random in mitotic cells. This may mean either that the antigen is released from tight complexes with DNA during mitosis, or that it remains associated at only a few regions of each chromosome and these regions are distributed randomly in chromosomes at mitosis. We believe the first possibility is more likely because the area of diffuse staining is quite large, covering the entire cell.

The antigen identified by AhNA-3 appears to be organized in the nucleus in a way different from the two discussed above. Its organization during G1 and G2 resembles that of a nuclear matrix. In addition, preparations of nuclear matrix contain the cognate antigens of AhNA 3 and AhNA 4. It is of interest to note that this structure disappears during mitosis, a period during which the breakdown of the nuclear envelope and presumably the nuclear matrix occurs. By early G1 the structure reappears. Apparently, therefore, the antigen is not bound to chromosomes at mitosis even though it clearly is a component of interphase chromatin. These results strongly suggest that the matrixlike structures visualized by antibodies AhNA-3 and AhNA-4 (Figs. 1 and 3) may constitute the nuclear matrix.

The contemporary view of chromosome structure and function is dominated by our considerable knowledge of the biochemical properties of histones and the high-mobility group of nonhistone proteins. This is largely because these proteins are abundant and can be isolated in native form. Furthermore, well defined functional assays are available for analysis of histones and HMG proteins. Bulk nonhistone chromosomal proteins are also thought to have important regulatory and structural roles. However, the regulatory proteins among these are probably present in much smaller amounts, and an almost total absence of suitable functional assays makes them quite refractory to conventional biochemical investigation. Thus, the immunological approach using monoclonal antibodies that we and others (38, 39, 41, 42) have begun will provide an effective method for accessing the functions of this important class of chromosomal proteins.

Indeed, our use of such antibodies to study changes in the distribution of specific nuclear antigens as HeLa cells traverse the cell cycle clearly indicates the advantage of the immunological approach.

This work was supported by National Institutes of Health (N.I.H.) grant GM 27236 to J. S. Bhorjee and N.I.H. grant GM 30211 to S. L. Barclay.

**REFERENCES**

1. Minsky, A. E., and H. Ris. 1951. The composition and structure of isolated chromosomes. J. Gen. Physiol. 34:473-492.
2. Odellhoch, H. H., B. M. Olivera, D. Tuan, and N. Davidson. 1967. Selective dissociation of histones from cell thymus nucleoprotein. J. Mol. Biol. 25:299-315.
3. Correns, D. E. 1978. Chromosomal organization of mitotic chromosomes. In The Cell Nucleus, H. B. Busch, editor. Academic Press, New York. 4:345-371.
4. Benesky, R., and D. S. Coffey. 1975. Nuclear matrix protein: association with newly synthesized DNA. Science (Wash. D.C.). 189:291-293.
5. Pederson, T., and J. S. Bhorjee. 1975. A special class of non-histone protein tightly complexed with template-inactive DNA in chromatin. Biochemistry. 14:5238-5242.
6. Bekhor, I. 1978. Reconstitution of chromatin. In The Cell Nucleus, H. B. Busch, editor. Academic Press, New York. 5:137-166.
7. Bekhor, I., and C. J. Minn. 1979. Simple isolation of DNA hydrophobically complexed with presumed gene regulatory protein (M). Biochemistry. 18:609-616.
8. Tsu, S.-Y., S. E. Harris, M.-J. Tsai, and B. W. O'Malley. 1976. Effects of estrogen on gene expression in chick oviduct. J. Biol. Chem. 251:4713-4722.
9. Norman, G. L., and I. Bekhor. 1981. Enrichment of selective active human gene sequences in the placental deoxyribonuclease acid fraction associated with tightly bound chromosomal proteins. Biochemistry. 20:3548-3578.
10. Chyi, T., and F. C. Schlager. 1971. Tissue differences in antigenic properties of non-histone protein-DNA complexes. Nature New Biol. (Lond.). 233:212-218.
11. Walbic, K., and L. S. Hnilica. 1973. The immunocomplex of nonhistone protein complexes with DNA. Nature New Biol. (Lond.). 242:153-155.
12. Jiao, F.-F., Y.-H. Tsai, K. Sakuma, and L. S. Hnilica. 1975. Regulation of in vitro mRNA transcription by a fraction of chromosomal proteins. J. Biol. Chem. 250:9431-9433.
13. Jagodzinski, L. L., J. C. Chilton, and J. S. Sevall. 1978. DNA-binding nonhistone proteins: DNA site association. Nucl. Acids Res. 5:1487-1499.
14. Wang, T. Y. 1978. The role of nonhistone chromosomal proteins in the interaction of prolate chromosomes with androgen receptor complex. Biochim. Biophys. Acta. 518:31-39.
15. Spelberg, T. C., R. Webster, G. Pifer, C. Thrall, and D. Wells. 1976. Role of nuclear proteins as high affinity sites ("acceptors") for progesterone in the avian oviduct. J. Steroid Biochem. 7:1091-1101.
16. Berzozky, R., and D. S. Coffey. 1977. Nuclear Matrix. Isolation and characterization of a framework structure from rat liver nuclei. J. Cell Biol. 73:616-637.
17. Adolph, K. W., S. M. Cheng, and U. K. Laemmli. 1977. Role of nonhistone proteins in metaphase chromosome structure. Cell. 12:803-816.
18. Eagle, H. 1959. Amino acid metabolism in mammalian cell cultures. Science (Wash. D.C.).
19. Bhotorje, J. S., and T. Pederson. 1973. Chromatin: its isolation from cultured mammalian cells with particular reference to contamination by ribonucleoprotein particles. Biochemistry. 12:2766-2773.

20. Bhotorje, J. S., and T. Pederson. 1972. Nonhistone chromosomal proteins in synchronized HeLa cells. Proc. Natl. Acad. Sci. USA. 69:2224-2228.

21. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (Lond.). 227:680-685.

22. Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA. 76:4350-4354.

23. McKearn, T. J., M. Sarmiento, A. Weiss, F. P. Stuart, and F. W. Fitch. 1979. Selective suppression of reactivity to rat histocompatibility antigens by hybridoma antibodies. In Lymphocyte Hybridomas. F. Melchers, M. Potter, and N. Warner, editors. Springer-Verlag, New York. 61-65.

24. Williams, A. F., G. Gallen, and C. Milstein. 1977. Analysis of cell surfaces by xenogeneic myeloma hybrid antibodies: differentiation antigens of rat lymphocytes. Cell. 12:663-673.

25. McLean, I. W., and P. K. Nakane. 1974. Periodate-lysine-paraformaldehyde fixative. A new fixative for immunoelectron microscopy. J. Histochem. Cytochem. 22:1077-1083.

26. Hngle, B., H. Guldner, F. A. Bautz, and A. Alonso. 1982. Cross-reaction of hnRNP-proteins of HeLa cells with nuclear proteins of Drosophila melanogaster demonstrated by a monoclonal antibody. Exp. Cell Res. 142:119-126.

27. Will, H., and E. K. F. Bautz. 1980. Immunological identification of a chromocenter-associated protein in polytene chromosomes. of Drosophila. Exp. Cell Res. 125:401-410.

28. Pederson, T. 1972. Chromatin structure and the cell cycle. Proc. Natl. Acad. Sci. USA. 69:2224-2228.

29. Karn, J., E. M. Johnson, G. Vidali, and V. G. Allfrey. 1974. Differential phosphorylation and turnover of nuclear acidic proteins during the cell cycle of synchronized HeLa cells. J. Biol. Chem. 249:667-677.

30. Moser, G. C., H. Muller, and E. Robbins. 1975. Differential nuclear fluorescence during the cell cycle. Exp. Cell Res. 91:73-78.

31. Moser, G. C., and H. Muller. 1979. Cell cycle dependent changes of chromosomes in mouse fibroblasts. Eur. J. Cell Biol. 19:116-119.

32. Natarajan, A. T., and A. Grupp. 1972. A fluorescence study of heterochromatin and nuclear organization in the laboratory and tobacco mouse. Exp. Cell Res. 74:245-250.

33. Fell, H. B., and A. F. Hughes. 1949. Mitosis in the mouse: a study of living and fixed cells in tissue cultures. Q. J. Microsc. Sci. 90:355-380.

34. Lambert, W. C., and G. P. Studzinski. 1969. Thymidine as a synchronizing agent. II. Partial recovery of HeLa cells from unbalanced growth. J. Cell Physiol. 73:261-266.

35. Thomas, D. B., and C. A. Lingwood. 1975. A model of cell cycle control: effects of thymidine on synchronous cell cultures. Cell. 5:37-42.

36. Yang, S.-J., G. M. Hahn, and M. A. Bagshaw. 1966. Chromosome aberrations induced by thymidine. Exp. Cell Res. 42:130-135.

37. Churchill, J. R., and G. P. Studzinski. 1970. Thymidine as synchronizing agent. III. Persistence of cell cycle patterns of phosphatase activities and elevation of nuclease activity during inhibition of DNA synthesis. J. Cell Physiol. 75:297-304.

38. Turner, B. M. 1981. Isolation of monoclonal antibodies to chromatin and preliminary characterization of target antigens. Eur. J. Cell Biol. 24:266-274.

39. Kane, C. M., P-F. Cheng, J. B. E. Burch, and H. Weintraub. 1982. Tissue-specific and species-specific monoclonal antibodies to avian red cell nuclear proteins. Proc. Natl. Acad. Sci. USA. 79:6265-6269.

40. Mayfield, J. E., L. A. Seranin, L. M. Silver, and S. C. R. Elgin. 1978. A protein released by DNase I digestion of Drosophila nuclei is preferentially associated with puff cells. J. Cell Biol. 104:539-546.

41. Howard, G. C., S. M. Abmaey, L. A. Shineld, V. L. Sato, and S. C. R. Elgin. 1981. Monoclonal antibodies against a specific nonhistone chromosomal protein of Drosophila associated with active genes. J. Cell Biol. 88:219-225.

42. Kuo, C-H., H. Gilson, A. B. Blumenthal, and J. W. Sedat. 1981. A library of monoclonal antibodies to nuclear proteins from Drosophila melanogaster embryos. Exp. Cell Res. 142:141-154.

43. Sanders, C., and E. W. Johns. 1974. A method for the large-scale preparations of two chromatin proteins. Biochem. Soc. Trans. (Lond.). 2:547-550.

44. Sausweber, P., M. Symmonsd, R. Kabocha, H. Will, and F. Bonhoeffer. 1980. Monoclonal antibodies against chromosomal proteins of Drosophila melanogaster. Chromosoma (Berl.). 80:253-275.