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Nucleoprotein Complexes from Metastatic Cells Containing Oncogenes and Tissue-Specific Genes: A Novel Method to Track Genes Associated with Specific Nucleoproteins

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ABSTRACT: Intact nuclei derived from murine metastatic large-cell lymphoma and human chronic myelogenous leukemia cells were digested to discrete subchromatin deoxyribonucleoprotein/ribonucleoprotein precursor complexes by treatment with *Msp*-I. The resultant complexes were composed of nucleoproteins (NPs) that were isolated and purified by two-dimensional isoelectric focusing/sodium dodecylsulfate polyacrylamide gel electrophoresis (2D-SDS-PAGE), electroelution from the gel, and removal of SDS by extractigel chromatography. Various NPs purified by 2D-SDS-PAGE were examined for the presence of oncogenes and tissue-specific genes using a dot-blot hybridization technique. The RNA polymerase products of NPs were labeled, purified, and subsequently used in a back-hybridization assay to identify transcripts for particular genes. By utilizing a 2D-SDS-PAGE Southwestern technique in parallel with the dot-blot and RNA back-hybridization assays, we assessed whether it is possible to "track" a gene and its associations in particular NPs. In patients with chronic myelogenous leukemia, we screened ~1000 NPs for *bcl*-2 sequences and found them present in a single NP of apparent $M_r \sim 19{,}000$, pI ~ 5.5. In murine RAW117-H10 cells transformed by the *abl* oncogene, we found by Western analysis that an antigen cross-reacting with *abl* antigen was localized to a *p53* gene-containing NP of apparent $M_r \sim 22{,}000$, pI ~ 7.2. A coincident Southwestern experiment using the same blot showed that the *abl* gene was bound by the same NP. The techniques described present the basis for "tracking" a particular gene to individual NPs and studying its relationship to other genes, their respective gene products, and its binding properties with particular NPs.

KEY WORDS: nucleoprotein, RNA polymerase, oncogene, gene expression, transcription, large-cell lymphoma, chronic mylogenous leukemia, metastasis.

I. INTRODUCTION

Eukaryotic gene regulation at the level of chromatin is poorly understood. Although a considerable amount of information has emerged during the last decade on *in vitro* transcription and the role of protein-DNA interactions at the DNA primary sequence level, in general, these studies have not yielded information about the transcriptional role of macromolecular associations within chromatin.[1–12]

Most conventional mammalian gene regulation studies use virus DNA models or other well-defined systems to assess protein-DNA gene regulatory interactions. Unfortunately, such models may be more analogous to macromolecular interactions involved in prokaryote gene regulation rather than those associated with regulation of eukaryotic genes. In eukaryotic systems, transcription does not occur on naked DNA. Therefore, investigators have focused on elucidating the nuclear matrix, nuclear scaffold,

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and radial loop organization of "active" and "inactive" chromatin domains. Such studies suggest that eukaryotic genes are anchored in the chromatin via pertinent nucleoprotein (NP) macromolecular associations.[13–17]

Particular chromatin regions in eukaryotes have been denoted by the presence of hypersensitive DNA sites, where portions of the DNA sequence are particularly accessible to digestion with DNase-I and/or *Msp*-I. These hypersensitive regions have been observed to be important in gene expression and chromatin structural organization.[18,19] Although certain portions of eukaryotic DNA are sensitive to specific endonuclease digestion, most of the DNA remains tightly associated with NPs within the chromatin structure and inaccessible to digestion with DNase-I and/or *Msp*-I.

In an effort to begin to assess individual gene and NP interactions in chromatin, we hypothesized that eukaryotic genes should have specific macromolecular associations or networks with distinct NPs defined by tight DNA associations (anchorage) within a particular subchromatin domain and its constituent NPs. To test this hypothesis, we developed a method to molecularly dissect the insoluble portions of the eukaryotic nucleus by direct nuclear digestion with *Msp*-I followed by fractionation using low ionic strength gel electrophoresis.[20–23] These *Msp*-I-derived subchromatin precursor complexes consisted of deoxyribonucleoproteins/ribonucleoproteins. The complexes were further fractionated into their constituent NPs by two-dimensional isofocusing sodium dodecylsulfate polyacrylamide gel electrophoresis (2D-SDS-PAGE).[23] The resultant NPs were then analyzed for *in vitro* enzyme activities by standard assays in conjunction with dot-blot hybridization assays to screen for the presence of various genes.[23]

We initially screened NPs from metastatic variants of the murine large-cell lymphoma cell line RAW117 for the presence of *abl, p53*, c-*neu*, c-H-*ras*, β-casein, 18s rDNA, and μ-chain immunoglobulin genes.[23] These studies were combined with RNA back-hybridization assays to determine whether individual NPs were able to synthesize mRNA *in vitro* that was capable of hybridizing to specific genes. In these studies, we also examined potential relationships between different NPs that could influence or regulate gene expression. For example, by including particular NPs purified from RAW117 nuclei in an NP reconstitution assay system, we found that an isolated NP of 140 kDa, pI ~ 5.8, was able to generate a mRNA that back-hybridized to β-casein DNA, although the β-casein gene is normally silent in RAW117 cells. This suggested that removal of the 140-kDa NP from its normal interactions in the nucleus resulted in release from silencing of the β-casein gene.[23]

To examine NP–NP interactions in terms of specific gene expression, we examined the *abl* oncogene and its tightly bound NP. In addition to the presence of *abl* gene sequences, we also found that the *abl* gene was bound by a specific NP in the RAW117 cell system. Using Western analysis, we were able to localize a cross-reacting antigen to the *abl* antigen in a single *p53* gene-containing NP of apparent M_r ~ 22,000 and pI ~ 7.2 from an NP precursor complex fraction.

This report also focuses on the use of a gene-tracking method to localize the *bcl*-2 gene in NPs isolated from the nuclei of chronic myelogenous leukemia cells. We found that the *bcl*-2 gene was localized to a single NP of apparent M_r ~ 19,000, pI ~ 5.2, in freshly isolated leukemia cells.

II. METHODS

A. Isolation of Subnuclear Chromatin Precursor NP Complexes and Purification of Constituent NPs

Subnuclear chromatin precursor complexes and smaller NP complexes were fractionated according to a previously described method involving direct digestion of the nucleus with *Msp*-I, followed by fractionation of precursor complexes by low ionic strength gel electrophoresis.[20–23] Precursor complexes were then incubated in the presence or absence of DNase-I according to our procedure, and constituent NPs were purified by 2D-SDS-PAGE. By this method, we were able to identify ~500 different NPs in the various precursor complexes (Figure 1). The individual NPs could be extracted from the 2D-SDS-PAGE gels,

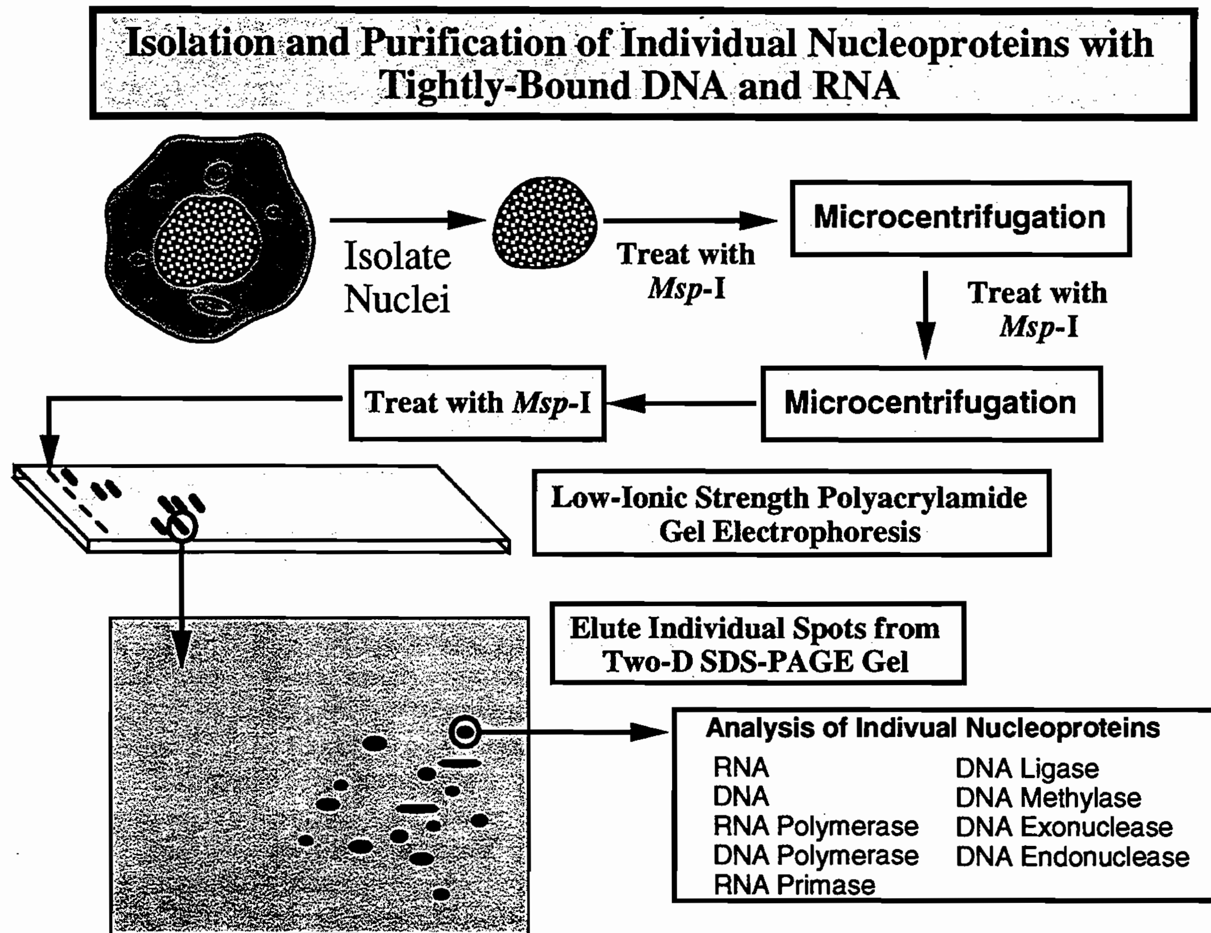


FIGURE 1. Methodological flow chart for the isolation and purification of individual NPs from eukaryotic cells. Nuclei are isolated and restriction digested with *Msp*-I, followed by a series of washes and another *Msp*-I digestion and washing. The resultant NP precursor complexes (S1, M1, S2, M2, 0.1K, and R) are fractionated by low ionic strength electrophoresis. Constituent NPs are analyzed by 2D-SDS-PAGE after incubation of the precursor NPs in the presence or absence of DNase-I. The resultant NPs are isolated and purified by excision from the 2D-SDS-PAGE gel, elution, and removal of SDS. Purified NPs are then analyzed for *in vitro* enzyme activities and screened for tightly associated gene sequence and respective mRNA products.

the SDS removed, and the NPs used for various assays as described below.[23]

B. Dot-Blot Hybridization Analysis

Identification of specific genes by dot-blot hybridization with purified NPs was performed using a method described by Pepin et al.[25] Prehybridization and subsequent hybridization with a *bcl*-2 gene probe was performed using Southern blot conditions as described by Maniatis et al.[26] The probe for *bcl*-2, an *Eco*RI-*Hin*DIII 2.8-kbp insert, was purchased from ONCOR and used at a concentration of 10 ng/μl. DNA probes were labeled with [α-^{32}P]dCTP (ICN, Irvine, CA) using a Random Primed DNA labeling Kit™ (Boehringer Mannheim, Indianapolis, IN). Posthybridization washing of the blots included the following washing steps: twice in 6×SSC-0.1% SDS for 20 min at 55°C; twice in 2×SSC-0.1% SDS for 20 min at 55°C; and a final rinse in 0.5×SSC-0.1% SDS at 55°C. Blots were dried and exposed to autoradiography.[23]

C. RNA Back-Hybridization Analysis

RNA back-hybridization analyses to identify synthesized RNA transcripts were performed with 2D-SDS-PAGE-purified NPs according to methods described by us.[23] Basically, *in vitro* RNA synthesis products from individual NPs and partial reconstitution of NP complexes were purified, radiolabeled, and used to probe back to immobilized cDNAs of a variety of genes. *In vitro* RNA synthesis was performed with purified NPs or a partially reconstituted NP complex consisting of several NPs at an NP concentration range of ~0.1 to 3 ng per reaction in TM buffer consisting of 0.01 m*M* Tris, 0.01 m*M* $MgCl_2$, 1 μg/μl bovine serum albumin, pH 7.8. Unlabeled ribonucleotides (rATP, rCTP, and rUTP) were added to each reaction at a final concentration of 0.1 ng. Radiolabeled [α-^{32}P]GTP was included in each reaction at a final concentration of 1 μCi (0.1 ng). Reactions were incubated at 37°C for 3 h. NP RNA synthesis products were purified by removal of unreacted [α-^{32}P]GTP by extra-cellulose™ desalting chromatographic columns (Pierce). Hybridization with the purified NP radiolabeled *in vitro* RNA synthesis products to Nytran-immobilized cDNAs was performed as previously described.[23]

D. Southwestern Analysis

Southwestern analysis[27] was performed according to a procedure described by Yan and Hung.[28] Two-dimensional isofocusing SDS-PAGE was performed on *Msp*-I-derived precursor subnuclear chromatin fractions as described previously.[23] The gels were then transferred to a 0.45-μm pore size nitrocellulose membrane in a buffer consisting of 48 m*M* Tris, 39 m*M* glycine, 1.3 m*M* SDS, 20% methanol, pH 7.0 for 1 h. Blocking of nonspecific associations was achieved by immersion in a 5% (w/v) nonfat dry milk in 10 m*M* Hepes, pH 7.9, for 1 h at room temperature, followed by incubation at room temperature for 1 h in hybridizing buffer (10 m*M* Hepes, pH 7.9, 50 m*M* NaCl, 10 m*M* $MgCl_2$, 0.1 m*M* EDTA, 1 m*M* dithiothreitol, 0.25% nonfat dry milk) containing 10,000 cpm of ^{32}P-labeled probe per milliliter. Following hybridization with a 1.5-kbp *Bgl* II fragment cDNA probe for the *abl* gene of the Abelson leukemia virus,[29] the nitrocellulose gel transfer was washed in four changes of hybridizing buffer containing 0.3 *M* NaCl for a period of 2 h, air dried, and autoradiographed.[23] An identical gel was cast for analytical purposes to assess the 2D-SDS-PAGE pattern for NPs incubated in the presence or absence of DNase-I. These gels were stained using Coomassie Blue, or they were silver stained.[23]

E. Western Analysis

Antibody against the *abl* antigen was generously provided by Dr. Ralph Arlinghaus (M. D. Anderson Cancer Center). The Western blot procedure was performed according to a method described by Gus et al.[30] Two-dimensional isofocusing SDS-PAGE gel blots were transferred to nitrocellulose (Hybond, ECL, Amersham) using a standard method.[31] To control for nonspecific interactions, the gel transfers were blocked for 1 h at room temperature in 0.15 *M* NaCl, 0.01 *M* Tris, pH 7.6, 0.1% Tween 20 (TBS/Tween), and 10% nonfat dry milk. After washing in TBS/Tween, the primary mouse monoclonal anti-*abl* antibody was diluted to 1:20,000 in TBS/Tween and incubated with the blot for 1 h at room temperature, followed by three washes for 15 min each in TBS/Tween. A peroxidase-tagged goat anti-mouse IgG second antibody (Amersham) diluted to 1:2000 was added, and incubation continued for 45 min at room temperature. After washing three times for 15 min each, the gel transfer was treated with the Enhanced Chemiluminescence (ECL) detection system using reagents supplied by Amersham and was autoradiographed.

III. RESULTS

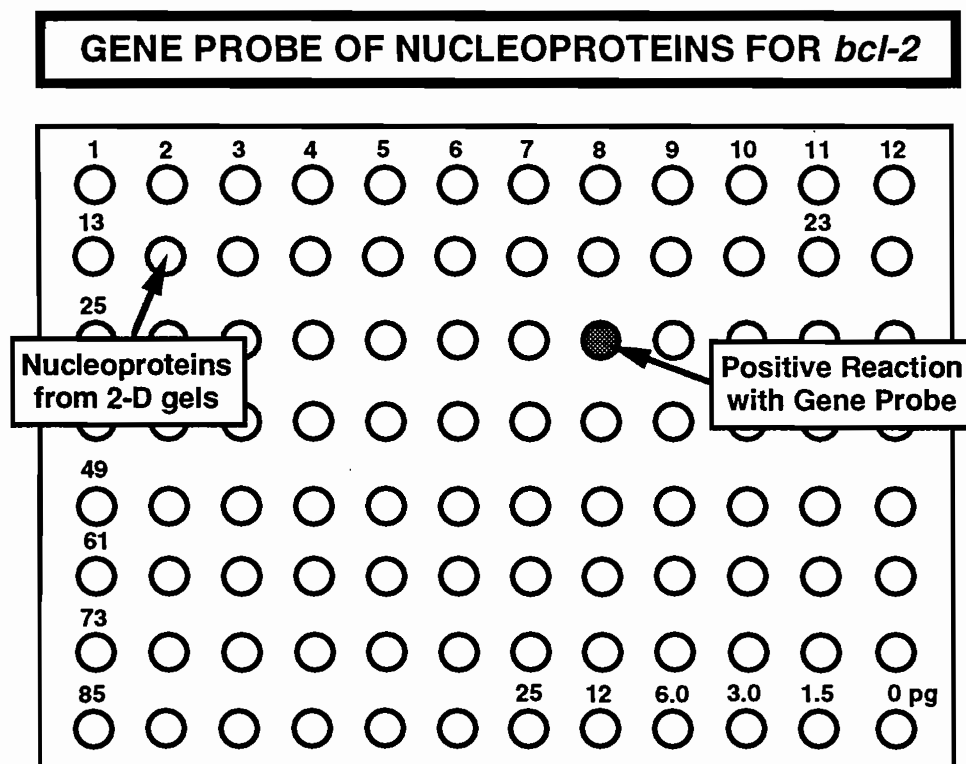
A. NP Purification Using 2D-SDS-PAGE Gels

Nuclei from a patient with chronic myelogenous leukemia and metastatic variants of the murine large-cell lymphoma RAW117 were

directly digested with *Msp*-I followed by a series of washes to release specific subnuclear chromatin precursor NP complexes. The resultant NP complexes were separated using low ionic strength electrophoresis, and the constituent NPs were subsequently purified to individual spots (or streaks) using 2D-SDS-PAGE (Figure 1).[22]

B. *Bcl*-2 Gene Localization in NPs from Chronic Myelogenous Leukemia Cells

Using a dot-blot hybridization method, we localized the *bcl*-2 gene to particular NPs isolated from the nuclei of a patient with chronic myelogenous leukemia. Two-dimensional isofocusing SDS-PAGE-purified NPs were dot-blotted to Nytran and subsequently subjected to hybridization according to the method of Pepin et al.[25] For example, incubation of the *bcl*-2 probe with 96 different individual NPs (Figure 2A)[23] resulted in a positive hybridization signal in only a single NP of apparent $M_r \sim 19{,}000$, pI ~ 5.5 (Figure 2B). To control for protein-protein interaction, the blot was stripped with 0.1 *M* NaOH, dried, and autoradiographed again. Since the autoradiography signal was not retained after NaOH stripping of the blot, we concluded that the initial dot-blot hybridization was due to DNA–DNA interactions. Similar studies have been performed using a variety of probes for tissue-specific genes and oncogenes with NPs from murine RAW117-H10 nuclei.[23] These results suggest that the presence of specific genes tightly associated with particular NPs is a common feature in chromatin.



A

FIGURE 2. Localization of *bcl*-2 gene sequences in NPs isolated from the nuclei of a patient with chronic myelogenous leukemia by dot-blot hybridization. (A) The template for the dot-blot hybridization protocol is illustrated for the assessment of 96 individual NPs. (B) Positive hybridization with *bcl*-2 gene probe is observed for a single NP of apparent $M_r \sim 19{,}000$, pI ~ 5.5.

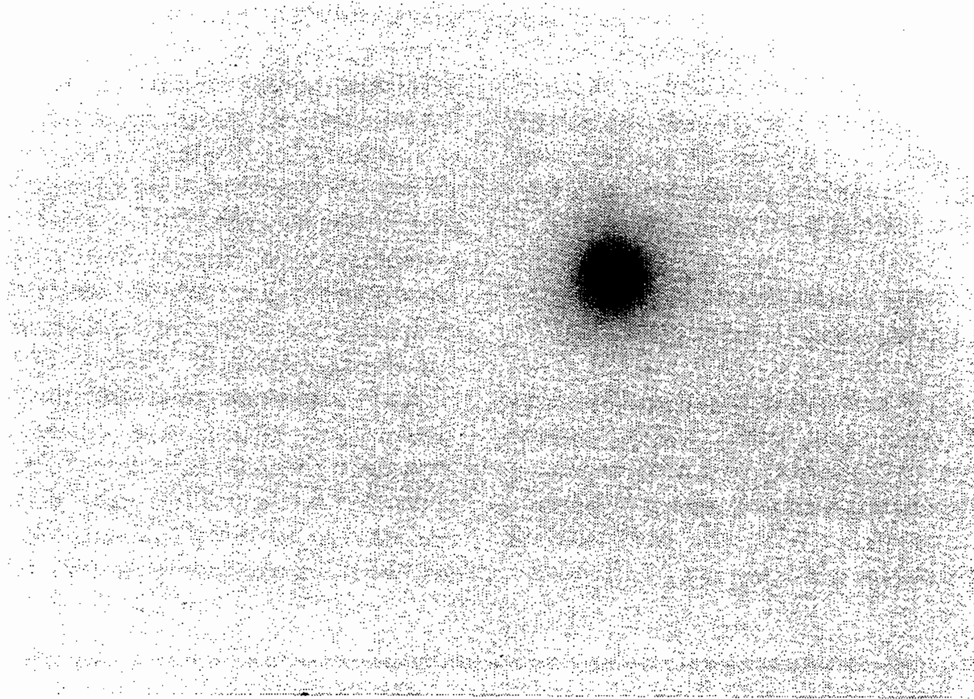


FIGURE 2B

C. Dot-Blot Analysis for *abl* Oncogene-Containing NPs from RAW117 Cells

Using the dot-blot technique, we previously identified NPs containing *abl* gene sequences in murine RAW117 large-cell lymphoma cells. The individual NPs from *Msp*-I-released NP complexes isolated from RAW117-H10 nuclei were purified by 2D-SDS-PAGE, extracted from the gel, blotted onto Nytran paper as in Figure 2A, and the blot was probed with an *abl* gene probe.[23] The results indicated that *abl* gene sequences were present in NPs of M_r ~ 120,000, pI ~ 5.8; M_r ~ 68,000, pI ~ 5.8; M_r ~ 57,000, pI ~ 5.2; M_r ~ 30,000, pI ~ 5.4; and M_r ~ 32,500, pI ~ 5.6.[23]

D. Southwestern Analysis for *abl* Oncogene-Binding NPs from RAW117 Cells

To further develop an approach to track individual genes with respect to particular mRNAs and gene protein products, we chose to investigate *abl* gene-binding to particular NPs by Southwestern analysis. Two-dimensional isofocusing SDS-PAGE was performed on *Msp*-I-released NP complexes from the large-cell lymphoma RAW117-H10 cell line. Prior to isofocusing, the NP subnuclear complexes from fraction 0.1K[23] were incubated at 37°C in the presence or absence of DNase-I for 3 h. Treatment with DNase-I enabled us to distinguish between NP complexes and individual constituent NPs that are loosely or tightly bound to NP complex DNA. A typical 2D-SDS-PAGE Coomassie Blue-stained NP pattern from an unsynchronized population of RAW117-H10 cells is shown for complex fraction 0.1K in Figure 3A. This DNase-I-treated complex NP fraction isofocusing pattern is characterized by a cluster of spots (streaks) in the apparent M_r range of ~22,000 to 17,000 with pIs in a range between 6.8 to 7.2. Southwestern analyses of this NP complex fraction indicated positive binding of the *abl* gene probe to a well-defined spherical portion of one of the spots of apparent M_r ~ 22,000, pI ~ 7.2.[23] This result suggests that *abl* recognizes protein sequences in this indi-

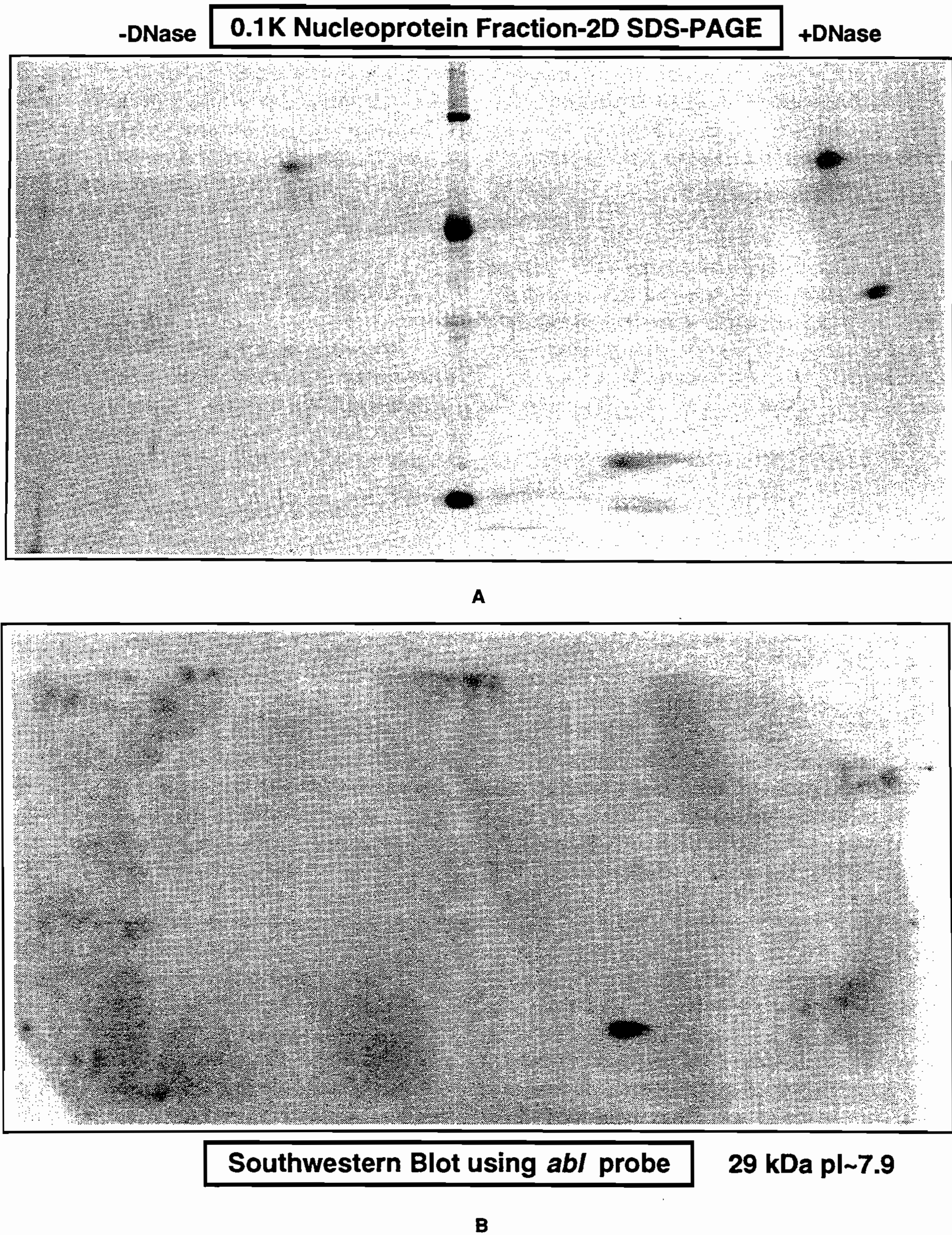


FIGURE 3. Analytical 2D-SDS-PAGE for NP precursor fraction 0.1K and its Southwestern analysis for *abl* gene-binding. (A) NP precursor fraction 0.1K was incubated in the presence or absence of DNase-I. The resultant Coomassie Blue-stained constituent NP pattern is shown for NP precursor complexes incubated with DNase-I (left-hand side of panel) or without DNase-I (right-hand side of panel). (B) Southwestern analysis for *abl* gene-binding indicates positive binding to a spherical portion of a streak of apparent M_r ~ 22,000, pI ~ 7.2.

vidual NP of apparent $M_r \sim 22{,}000$, pI ~ 7.2, and Western analysis of the same blot used for Southwestern analyses indicated that an antigen cross-reactive with *abl* antigen is localized to the same NP (data not shown). Previously, we observed that the *abl* oncogene was not inherently associated with this particular NP. However, using a dot-blot hybridization assay with purified RAW117 NPs, we did observe the presence of tightly bound *p53* gene in this same NP.[23] Therefore, we tentatively conclude that in this system the *abl* antigen or a cross-reacting antigen can be tracked in terms of its association with an NP containing *p53* gene sequences.

E. RNA Back-Hybridization Studies for the β-Casein Gene in RAW117-H10 Cells

RNA back-hybridization studies were performed with an individual 2D-SDS-PAGE purified-NP of apparent $M_r \sim 140{,}000$, pI ~ 5.8, and NP complex reconstitutes containing several purified NPs. Some NP complexes, and in rare cases large individual NPs, were capable of synthesizing RNA products using a standard *in vitro* RNA synthesis assay.[23] After synthesis, the RNA products were purified, radiolabeled, and used to probe cDNAs of genes that had been immobilized on Nytran. We observed that an individual NP of apparent $M_r \sim 140{,}000$, pI ~ 5.8, produced β-casein mRNA in the RNA synthesis assay. However, when the NP of $M_r \sim 140{,}000$, pI ~ 5.8, was reconstituted with NPs of $M_r \sim 120{,}000$, pI ~ 5.8, and $M_r \sim 57{,}000$, pI ~ 5.2, β-casein mRNA synthesis was inhibited concomitant with an increase in the synthesis of mRNAs that hybridized to c-H-*ras* and c-*neu* oncogenes (Figure 4). If other NPs were reconstituted with the NP of apparent $M_r \sim 140{,}000$, pI ~ 5.8, mRNAs of unknown origin were synthesized. It was possible to inhibit the synthesis of c-*neu* mRNA while retaining the synthesis of c-H-*ras* mRNA merely by substituting a DNase-I-treated NP of apparent $M_r \sim 140{,}000$, pI ~ 5.8, in the NP reconstitute with NPs of apparent $M_r \sim 57{,}000$, pI ~ 5.2, and M_r 120,000, pI ~ 5.8 (Figure 4). Thus subtle substitution of NPs in the assay can alter the expression of specific mRNAs. However, an even more significant observation is that substitution of particular NPs can promote the *in vitro* expression of the β-casein gene, which normally is silent in RAW117 cells but is expressed when the specific NP of apparent $M_r \sim 140{,}000$, pI ~ 5.8, is removed from the influence of other NPs in an NP complex.[23] A model is shown for the *in vitro* turning on and off of β-casein gene expression using the NP assay (Figure 5).

IV. DISCUSSION

The basic underlying premise for the studies presented in this report evolved from observations that the active genes in eukaryotic systems are tightly associated, or anchored, to nuclear matrix or scaffolding structures within the nucleus.[14–16] We developed a technique to selectively dissect the nucleus into subchromatin NP complex domains[22] followed by analyses of the constituent NPs.[23] These NPs were purified to individual spots or streaks on 2D-SDS-PAGE gels followed by excision and removal of SDS to obtain NPs that were active in a variety of enzyme assays.[23] For example, we showed that some of the NPs appear to be multifunctional and capable of RNA polymerase and other activities. By mixing particular NPs to form reconstituted NP complexes, we were able to either inhibit or promote the synthesis of specific mRNA products *in vitro*.[23] In the example used here, we found that the normally silent β-casein gene in the large-cell lymphoma cell line RAW117 can be expressed during an *in vitro* RNA polymerase assay using only a large, isolated NP of apparent $M_r \sim 140{,}000$, pI ~ 5.8, as the endogenous template. This gene could again be silenced by addition of different NPs to the NP mixture, suggesting that the silencing of the β-casein gene in these cells may involve specific NP–NP interactions in chromatin domains (Figure 5).

To support the hypothesis of Mirkovitch et al.[16] that genes may be tightly bound into specific chromatin domains, we attempted to screen individual 2D-SDS PAGE-purified NPs for the presence of genes that could not be disassociated by boiling or treatment with SDS and urea. Our idea was that some genes are tightly bound to specific NPs and that their arrangements within chromatin domains might yield unique macromolecular

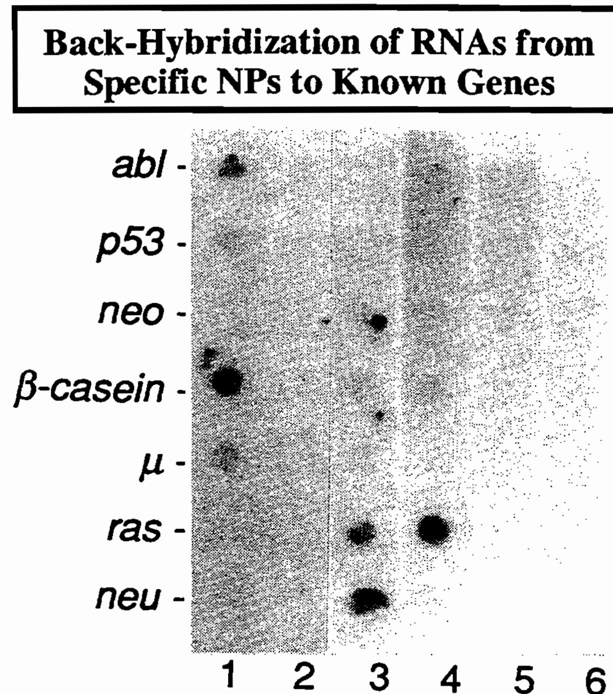


FIGURE 4. Back-hybridization analysis for the synthesis of specific mRNAs. RNA products from an individual NP of apparent M_r ~ 140,000, pI ~ 5.8, that contains the β-casein gene and various partial NP reconstitutes indicate that the *in vitro* differential expression of β-casein, c-H-*ras,* and c-*neu* mRNAs is contingent on the presence of specific NPs in the assay. Aliquots of cDNAs for *abl, p53, neo* (a negative control), β-casein, μ-chain immunoglobulin gene, c-H-*ras,* and c-*neu* genes were spotted onto strips of Nytran and subjected to RNA back-hybridization using mRNAs generated with specific NPs or combinations of NPs. Lane 1, RNA synthesized from a single NP of apparent M_r ~ 140,000, pI ~ 5.8, showed positive hybridization to the β-casein gene. Lane 2, When a single NP of apparent M_r ~ 31,000, pI ~ 7.7, was used, no RNA hybridization was detected for the genes used in this screen. Lane 3, reconstituting the NP of apparent M_r ~ 140,000, pI ~ 5.8, with NPs of apparent M_r ~ 57,000, pI ~ 5.2, and M_r ~ 120,000, pI ~ 5.8, demonstrated positive RNA hybridization to c-H-*ras* and c-*neu* genes but not the β-casein gene. Lane 4, The detection of mRNA to c-*neu* was obliterated by substituting a DNase-I-treated NP of M_r ~ 140,000, pI ~ 5.8, reconstituted with the same NPs as shown in lane 3. Lane 5, Hybridization of RNA to the genes in the screen was not detected when the NP of apparent M_r ~ 140,000, pI ~ 5.8, was reconstituted with an NP of apparent M_r ~ 30,000, pI ~ 5.4. Lane 6, A reconstitution consisting of NPs of apparent M_r ~ 34,000, ~35,000, and ~16,000 with respective pI ~ 5.5, ~5.2, and ~8.0 did not synthesize RNAs to the genes in the screen.

patterns contingent upon the inherent nature of the cell and state of its nucleus. In fact, very few of the NPs purified by our methodology retained tightly bound gene sequences under the conditions used for isolating individual NPs. However, we were able to find *p53, abl,* c-H-*ras,* c-*neu,*

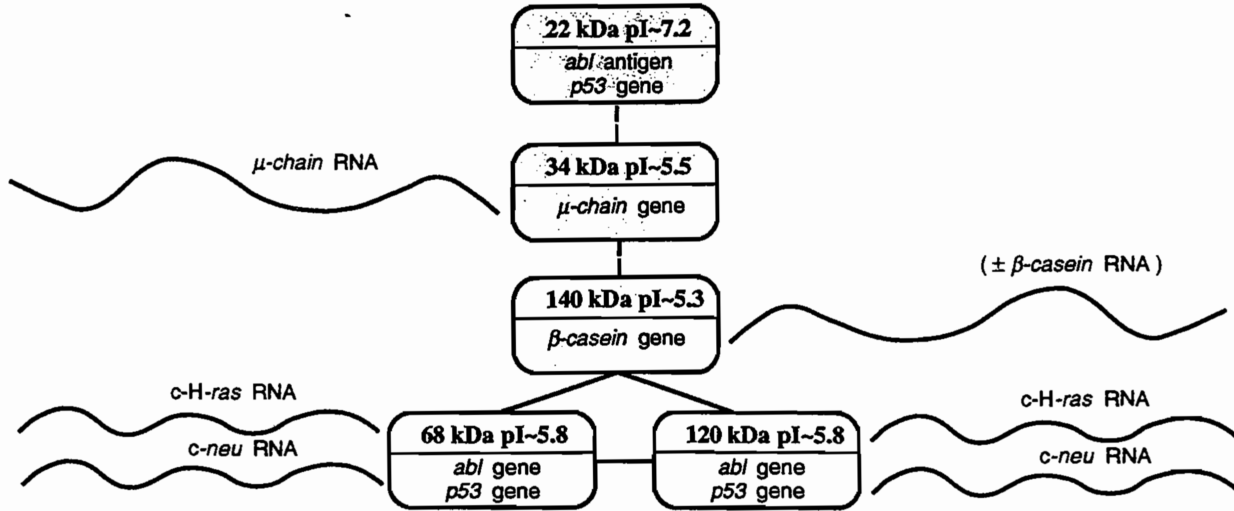


FIGURE 5. A model for NP regulation of β-casein gene expression. Putative NP–NP interactions are illustrated to demonstrate the differential effects of various specific NPs on the *in vitro* synthesis of β-casein, c-H-*ras*, c-*neu* mRNAs from Figure 4. When an NP of apparent M_r ~ 140,000, pI ~ 5.8, is used individually or reconstituted with other NPs in the RNA synthesis assay, different patterns of RNA synthesis are found, suggesting that NPs can affect the regulation of specific genes.

β-casein, 18s rDNA, and μ-chain immunoglobulin gene sequences in particular NPs in the metastatic RAW117 large-cell lymphoma cell system.[23] Data presented here also indicate that we could localize *bcl*-2 gene sequences to specific NPs from a patient with chronic myelogenous leukemia, in this case to only one individual NP of apparent M_r ~ 19,000, pI ~ 5.5. We used this technique in other cell lines and tissues and were successful in localizing genes such as π- and μ-glutathione-s-transferase to specific NPs (unpublished observations). We concluded, therefore, that the utilization of our method designed to purify NPs by 2D-SDS PAGE is applicable to a number of different species and cell systems. The ability to localize specific gene sequences to individual NPs provides us with a powerful tool for tracking genes at the subchromatin level and for deducing possible interrelationships of genes to other genes, their mRNAs, and their respective gene products.

In this report, we used for the first time Southwestern 2D-SDS-PAGE analyses of NP complexes (untreated or treated with DNase-I) to demonstrate that the *abl* gene can recognize protein sequences in an individual *p53* gene containing NP of apparent M_r ~ 22,000, pI ~ 7.2, isolated from RAW117 nuclei. We wish to point out, however, that these data are only an initial analysis of one NP complex precursor fraction and do not preclude *abl* gene binding by other NPs. When combining this observation with observations made by DNA dot-blot hybridization and RNA back-hybridization, it is possible to track the *abl* gene, *abl* gene-binding NPs, and *abl* or cross-reacting by antigen with respect to other genes in RAW117-H10 nuclei. It is interesting that the *abl* gene was bound to an NP that contains tightly associated *p53* gene sequences. Since the *p53* gene encodes a tumor-suppressor protein, we hypothesize that the macromolecular relationships involved in this NP could be pertinent to gene regulation, at least in this *in vitro* system. We speculate, based on our observations that the tissue-specific μ-chain immunoglobulin gene is organized in a different manner with respect to the *abl* and *p53* genes in NPs isolated from the poorly metastatic RAW117-P large-cell lymphoma cell line and various clonal metastatic variants,[23] that

differences in oncogene, tumor-suppressor gene, and tissue-specific genes and their interactions may be important in gene regulation and molecular processes involved in determining the phenotypic state of a cell.

If one considers a network of genes as being defined by particular NP–NP macromolecular interactions, it may become feasible in the future to relate particular genes and NPs to specific states of chromatin organization and cellular regulation. We speculate that eukaryotic genes may even communicate with each other, their encoded mRNAs, and their gene products via subchromatin NP networks. Thus, the pattern of NP macromolecular associations and the presence of tightly bound, tissue-specific genes and oncogenes may reflect a blueprint for eukaryotic chromatin organization that could contribute to the regulation of gene expression and phenotype of the cell. Such an NP–NP network model encompassing NPs and their tightly bound gene sequences and their possible involvement in silencing or promoting mRNA synthesis of β-casein, c-H-*ras*, and c-*neu* mRNAs in RAW117 cells is depicted in Figure 5. This oversimplified model involving only a few NPs and their associations probably under-represents to a great degree the types of complex interactions that occur between NPs in eukaryotic chromatin.

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