Journal of Cellular Biochemistry Supplements 32/33:158–165 (1999)

Nucleoprotein Gene Tracking: Localization of Specific HIV-1 Genes to Subchromatin Nucleoprotein Complexes Containing Endonuclease Activity in HIV-1-Infected Human Cells

Nancy L. Nicolson and Garth L. Nicolson*

Institute for Molecular Medicine, Huntington Beach, California 92649

Abstract We developed a technique with which to isolate specific subchromatin deoxyribonucleoprotein/ribonucleoprotein precursor complexes containing discrete genes from intact mammalian nuclei by direct restriction enzyme treatment with *Msp*I. These nucleoprotein complexes can be further fractionated and purified by two-dimensional isoelectric focusing/sodium dodecyl sulfate-polyacrylamide gel electrophoresis. After electroelution and removal of detergent, virtually thousands of nucleoprotein complexes can be examined for the presence of tightly bound genes and enzymatic activities. Nucleoprotein gene tracking procedures were applied to study the acidic nucleoprotein complexes from steady-state human H9 cells uninfected or infected with human immunodeficiency virus type 1 (HIV-1) virus. The purified nucleoprotein complexes were screened for the presence of loosely and tightly associated HIV-1 gene sequences (*pol*, *env*, *tat*, and *rev*) using a DNA hybridization protocol. In HIV-1-infected cells, four specific nucleoprotein complexes out of several hundred were found to contain tightly bound HIV-1 *pol* gene sequences after purification. By contrast, the other HIV-1 gene sequences (*env*, *tat*, and *rev*) were not tightly bound to any of the nucleoprotein complexes in HIV-infected cells. The observations suggest that certain HIV-1 genes associate with specific chromatin nucleoprotein complexes, regardless of their pattern of DNA integration into the human genome. At least two of the HIV-1 *pol*-containing nucleoprotein complexes of apparent M_r ~94,000, pI ~6.5, and M_r ~47,000, pI ~5.1 contain DNA endonuclease activity. This was confirmed in the present study, using linearized pUC19 plasmid substrate. The technique can be used to study a variety of problems concerning the association of specific genes and enzymes with specific nucleoprotein complexes J. Cell. Biochem. Suppls. 32/33:158–165, 1999.

Key words: nucleoprotein complexes; HIV-1 genes; restriction enzyme; *Msp*I; H9 cells; HIV-1 polymerase; gene hybridization; endonuclease

Specific chromatin regions important in gene expression and chromatin structural organization have been identified by the presence of hypersensitive DNA sites that are particularly accessible to digestion with either DNase-I or *Msp*I, or both [Nichols and Felsenfeld, 1983; Emerson and Felsenfeld, 1984]. Although certain portions of eukaryotic DNA are sensitive to specific endonuclease digestion, most DNA remains tightly associated with nucleoprotein complexes (NPC) within chromatin and inaccessible to digestion with DNase-I and/or *Msp*I.

To assess individual gene and NPC interactions in chromatin, we have used these observations to develop a method for molecular dissection of the insoluble portions of the eukaryotic nucleus by direct digestion of intact nuclei with *Msp*I [Rosenberg-Nicolson and Nicolson, 1992a,b; Nicolson and Nicolson, 1994a,b]. The *Msp*I-derived subchromatin complexes consisted of deoxyribonucleoproteins/ribonucleoproteins (DNP/RNP) displaying enzymatic activity. The DNP/RNP complexes can be further purified into their constituent NPC by two-dimensional isofocusing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (2D SDS-PAGE). After removal of detergent, the resultant

Abbreviations used: 2D SDS-PAGE, two-dimensional isoelectric focusing/sodium dodecylsulfate polyacrylamide gel electrophoresis; DNP/RNP, deoxyribonucleoproteins/ribonucleoproteins; HIV-1, human immunodeficiency virus-1; NPGT, nucleoprotein gene tracking; NPC, nucleoprotein complexes.

*Correspondence to: Garth L. Nicolson, Office of the Chief Scientific Officer, Institute for Molecular Medicine, 15162 Triton Lane, Huntington Beach, CA 92649. E-mail: gnicimm@ix.netcom.com

Received 31 August 1999; Accepted 2 September 1999

NPC can be analyzed for in vitro enzymatic activity by standard assays in conjunction with DNA hybridization assays, to screen for the presence of various genes [Nicolson and Nicolson, 1993, 1994a,b]. We previously found that specific NPC contained certain tightly bound genes or gene sequences and that some contained specific enzymatic activities, such as DNA polymerase, RNA polymerase, and RNA primase [Rosenberg-Nicolson and Nicolson, 1992a,b]. The technique of isolating specific NPC and their analyses for the presence of specific genes and enzymatic activities has been termed nucleoprotein gene tracking (NPGT) [Nicolson and Nicolson, 1994a]. This technique has been used for a variety of purposes. This article illustrates its usefulness in identifying the association of HIV-1 genes with specific NPC that contain enzymatic activity.

MATERIALS AND METHODS

Cells

For the experiments, we used H9 cells that had been infected with HIV-1 [Aldovini et al., 1986]. Nuclei were harvested 7 days after the uninfected or HIV-1-infected cells had reached steady-state growth. NPC were derived by previously described procedures involving direct sequential restriction digestion of purified nuclei with *Msp*I, partial fractionation of the chromatin complexes into six overlapping fractions (fractions S1, S2, M1, M2, 0.1K, and R) by low-ionic-strength gel electrophoresis, followed by isofocusing 2D SDS-PAGE to further purify NPC (Fig. 1).

Isolation of Subnuclear Chromatin Precursor NPC and Purification of Nucleoproteins

Subnuclear chromatin precursor complexes and smaller NPC were fractionated according to a previously described method involving direct digestion of purified, intact nuclei with *Msp*I, followed by fractionation of precursor complexes by low-ionic-strength gel electrophoresis according to Nicolson and Nicolson [1994a,b]. Precursor complexes were then incubated in the presence or absence of DNase-I according to our procedure, and constituent NPC were purified by isofocusing 2D SDS-PAGE. With this method, we were able to identify ~1,000 different NPC in the various precursor complexes. The individual NPC could be extracted from the 2D SDS-PAGE gels, the SDS

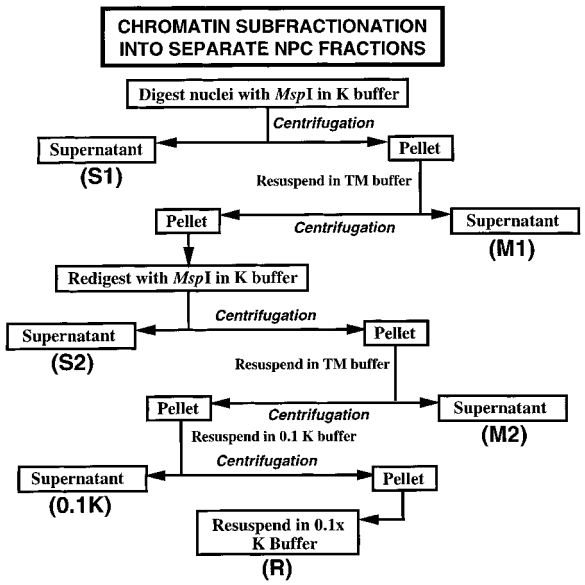

Fig. 1. Flow chart depicting steps in the purification of *Msp*I-derived subnuclear nucleoprotein complex (NPC) fractions and NPC constituents. Nuclei from steady-state human immunovirus type 1 (HIV-1)-infected H9 cells were digested according to previously described procedures to generate six subnuclear chromatin complex fractions (S1, M1, S2, M2, 0.1K, and R).

removed, and the NPC used for various assays as described below.

DNA Hybridization Analysis

Identification of specific genes by slot-blot hybridization with purified NPC was performed using a method described by Pepin et al. [1990]. The HIV-1 *pol* probe, a 41-oligomer with the sequence TAG TAG CCA GCT GTG ATA AAT GTC AGC TAA AAG GAG AAG CC, was purchased from Synthetic Genetics (San Diego, CA), as was the probe for HIV-1 *env* gene, a 33-mer of sequence AC G GTA CAG GCC AGA CAA TTA TTG TCT GGT ATA. The HIV-1 *tat* probe, a 320-bp complementary DNA (cDNA), was provided by Dr. Andrew Rice (Baylor College of Medicine, Houston, TX). The HIV-1 *rev* probe, a 350-bp *Nco*I-*Xho*I cDNA, was provided by Dr. Wade Harper (Baylor College of Medicine, Houston, TX). Prehybridization and subsequent hybridization with specific gene probes were performed using Southern blot conditions as described by Maniatis et al. [1982]. 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 [Nicolson and Nicolson, 1994a,b]. For certain gene probes, the hybridization and washing conditions were optimal in 6× SSC-0.1% SDS for 20 min at 60°C, and a final rinse in 0.5× SSC-0.1% SDS at 60°C. Blots were dried and exposed to autoradiography [Nicolson et al., 1998a].

DNA Endonuclease Activity

A standard DNA endonuclease in vitro assay was conducted using *Eco*RI-linearized pUC19 plasmid as the substrate. We examined the four 2D SDS-PAGE-purified HIV-1 *pol* gene-positive NPC of apparent M_r ~31,000, ~40,000, ~47,000 and apparent pI ~8.7, ~5.0, ~4.9 and ~6.5, respectively, from fraction M2 and one NPC of apparent M_r of ~94,000, pI ~6.5 from fraction R plus control NPC that did not contain HIV-1 *pol*. The reaction mixture contained 200 ng of ^{32}P-labeled (random) pUC19 plasmid linearized by treatment with *Eco*RI in a total volume of 15 µl of buffer (0.05 M Tris-HCl, 0.01 M $MgCl_2$, 0.005 M KCl, pH 7.6). Incubation of NPC with linearized pUC19 was performed at 37°C for 2 h. Samples were than analyzed by standard DNA flat-bed electrophoresis for 2 h (0.8% agarose in 0.04 M Tris-acetate, 0.001 M EDTA) using a mini-gel apparatus. The gel was subsequently soaked in solution (1.5 M NaCl, 0.5 M NaOH) for 1 h and transferred to nitrocellulose by capillary transfer in 10× SSC. After drying, the transfer was exposed to x-ray film. Relatively few (<1%) of NPC from HIV-1-infected H9 cells demonstrate endonuclease activity using this assay.

RESULTS

Localization of HIV-1 Genes to Specific NPC Fractions

The NPGT technique has been used to study infection of cells by viruses and microorganisms. As an example of this approach we chose to study the infection of human cells by the human immunodeficiency viruses, such as HIV-1, to learn more about the site(s) of incorporation and/or NPC association of the HIV-1 genes in the human genomes of infected cells. Although considerable progress has been made in understanding the events that occur during HIV-1 infection of human cells (Varmus, 1988; Berkhout and Jeang, 1989; Braddock et al., 1989; Cullen and Greene, 1989; Dingwell et al., 1989; McNearney et al., 1992; Levy, 1993; Asante and Skalka, 1997], little is known about HIV-1 infection in terms of nuclear cytological events, such as the association of HIV-1 genes with chromatin NPC after their integration into, or tight association with, the host genome. Nor is it known whether HIV-1 genes are associated with specific NPC and how this might affect the functional organization and enzymatic activities of chromatin.

Our hypothesis is that HIV-1 genes associate with specific NPC and their constituents after HIV-1 integration into the host genome. The cell system we used to study integration of HIV-1 genes and their association with specific NPC is based on a human T-cell line (H9) infected with HIV-1 [Varmus, 1988]. Human H9 nuclei were harvested 7 days after mock infection or when HIV-1-infected cells had reached steady-state growth. NPC were derived by the procedures described above involving direct sequential restriction digestion of intact H9 nuclei with *Msp*I, partial purification of the chromatin complexes into six overlapping fractions by low-ionic strength gel electrophoresis, followed by 2D SDS-PAGE separation for further purification of the NPC components. (Figure 2 shows one such fraction [R] separated by 2D SDS-PAGE.) Mock-infected H9 cells were used as NPC controls. Using these procedures, we previously described that certain genes (or gene sequences) could be removed from the NPC by treatment with 6 M urea-1% SDS; these were operationally defined as loosely NPC-associated genes. The genes that remained after this treatment were defined as tightly associated genes [Nicolson and Nicolson, 1994a].

After *Msp*I treatment of HIV-1-infected H9 cells, HIV-1 *pol* gene sequences were found in tight association with NPC fractions M2 and R (Fig. 3), along with loosely associated HIV-1 *rev* gene sequences, because treatment with urea-SDS removed *rev*, but not *pol* gene sequences from these NPC fractions (data not shown). HIV-1 *pol* was identified in NPC fraction M2 associated with NPC components of apparent M_r ~31,000, ~40,000, ~47,000 and apparent pI of ~8.7, ~5.0 and ~5.1, respectively (Fig. 3B). By contrast, the HIV-1 *tat* gene sequences were detected in loose association with NPC fractions M1, M2, 0.1K, and R (data not shown). These results suggest that, with the exception

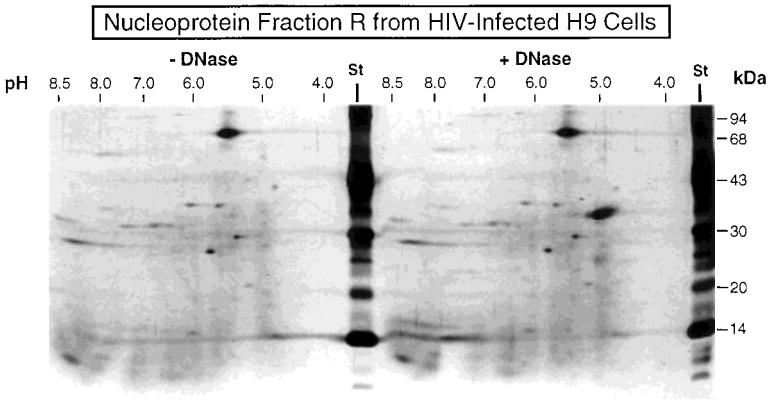

Fig. 2. Subnuclear nucleoprotein (NPC) fractions that were separated by low-ionic-strength gel electrophoresis were further fractionated by two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (2D SDS-PAGE) in the presence (+DNase) or absence (−DNase) of DNase-I treatment.

of the *pol* gene, most of the HIV-1 genes or gene sequences are loosely associated with host chromatin nucleoproteins. Previously we found that the tumor suppressor gene *p53* and the 18S ribosomal DNA (rDNA) gene or gene sequences were present in NPC fractions M1 and M2 of murine large-cell lymphoma cells [Rosenberg-Nicolson and Nicolson, 1992a; Nicolson and Nicolson, 1994a,b]. In addition, of the HIV-1 genes we detected, the *pol* gene in tight association with the topographically buried NPC fraction R, and after purification of the NPC components from fraction R by 2D SDS-PAGE, the *pol* gene was found to be tightly associated with one NPC component of apparent M_r ~94,000 and apparent pI ~6.5 (Fig. 3A). Further treatment of these NPC with DNase-I did not release the *pol* sequence. We have not found detectable amounts of HIV-1 *gag* or *env* in the equivalent NPC fractions (Fig. 3C). We recently found that the HIV-1 *env* and *gag* genes are tightly associated with several NPC components found in the cytoplasm of HIV-1-infected H9 cells (data not shown).

Endonuclease Activity

We examined the four NPC that contained tightly bound *pol* gene sequences and other NPC that did not contain tightly bound HIV-1 gene sequences. Of the NPC examined, only two were found to have endonuclease activity in the pUC19 assay. One, of apparent M_r ~94,000 and apparent pI ~6.5, was from fraction R, and one (apparent M_r ~47,000 and apparent pI ~5.1) was from fraction M2 (Fig. 4). None of the other NPC contained endonuclease activity in the pUC19 assay.

DISCUSSION

NPGT has been used to analyze NPC fractions from various cell systems for the presence of specific genes as well as enzymatic activity. For example, we have screened NPC from metastatic variants of the murine large cell lymphoma for the presence of *abl*, *p53*, c-*neu*, c-H-*ras*, β-casein, 18S rDNA, and μ-chain immunoglobulin genes and found that these genes were differentially expressed in the highly metastatic variants [Rosenberg-Nicolson and Nicolson 1992b; Nicolson and Nicolson, 1992b, 1993,1994a,b; Nicolson et al., 1995; 1996]. We also used RNA back-hybridization assays to determine whether individual nuclei-derived NPC are capable of synthesizing mRNA in vitro that could back-hybridize to specific genes, indicating the presence of enzymatic activities capable of producing RNA with specific sequences capable of hybridizing back to the gene sequences from which they were derived. In these studies, we found that a small subset of different lymphoma-derived NPC could influence or regulate gene expression of NPC that contained RNA polymerase activity. These NPC could synthesize a messenger RNA (mRNA) transcript after addition of the appropriate precursors and cofactors for RNA synthesis. For example, by including particular NPC purified from lymphoma nuclei in a NPC reconstitution assay, we found that an isolated NPC 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 these lymphoma cells. This finding suggested that removal of a specific NPC from their normal interactions in the nucleus resulted in release from silencing of the

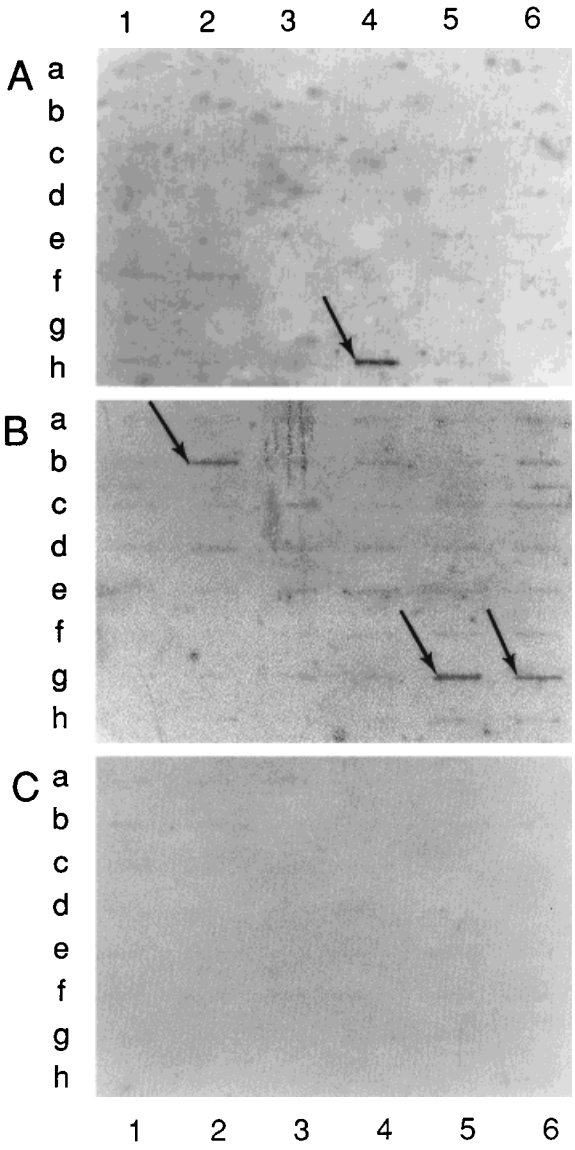

Fig. 3. Slot-blot hybridization analyses for the presence of human immunodeficiency virus type 1 (HIV-1) *pol* and *env* in two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (2D SDS-PAGE)-purified nucleoprotein complex. Purified NPC components were removed from the 2D SDS-PAGE gel, slot-blotted on Nytran paper, hybridized with ^{32}P-labeled gene probes, and subjected to autoradiography. Southern analyses were performed under moderate stringency conditions. Autoradiography was performed for 14 days at −70°C. The 2D SDS-PAGE-purified NPC were analyzed for the presence of HIV-1 *pol* gene sequences. **A:** Fraction R. Row 1: a, M_r ~30,000, pI ~5.2; b, M_r ~26,000, pI ~6.2; c, M_r ~19,000, pI ~5.1; d, M_r ~18,000, pI ~6.5; e, M_r ~15,000, pI ~8.1; f, M_r ~12,000, pI ~8.6; g, M_r ~12,000, pI ~5.6; h, M_r ~96,000, pI ~5.8. Row 2: a, M_r ~28,000, pI ~5.7; b, M_r ~27,000, pI ~5.1; c, M_r ~20,000, pI ~5.6; d, M_r ~17,000, pI ~5.6; e, M_r ~14,000, pI ~8.2; f, M_r ~12,000, pI ~8.2; g, M_r ~11,000, pI ~6.0; h, M_r ~94,000, pI ~5.8; Row 3: a, M_r ~28,000, pI ~5.6; b, M_r ~28,000, pI ~4.8; c, M_r ~22,000, pI ~8.4; d, M_r ~19,000, pI ~5.0; e, M_r ~14,000, pI ~8.5; f, M_r ~13,000, pI ~7.0; g, M_r ~10,000, pI ~5.6; h, M_r ~68,000, pI ~5.8. Row 4: a, M_r ~29,000, pI ~5.1; b, M_r ~26,000, pI ~5.6; c, M_r ~25,000, pI ~8,6; d, M_r ~15,000, pI ~8.4; e, M_r ~14,000, pI ~6.6; f, M_r ~12,000, pI ~7.0; g, M_r ~11,000, pI ~7.6; h, *M_r ~94,000, pI ~6.5. Row 5: a, M_r ~27,000, pI ~8.5; b, M_r ~23,000, pI ~4.8; c, M_r ~21,000, pI ~8.5; d, M_r ~17,000, pI ~8.6; e, M_r ~14,000, pI ~5.8; f, M_r ~10,000, pI ~7.0; g, M_r ~10,000, pI ~8.3; h, M_r ~94,000, pI ~7.5. Row 6: a, M_r ~27,000, pI ~8.2; b, M_r ~24,000, pI ~5.1; c, M_r ~19,000, pI ~8.7; d, M_r ~16,000, pI ~7.2; e, M_r ~14,000, pI ~4.9; f, M_r ~13,000, pI ~6.0; g, M_r ~9,000, pI ~8.4; h, blank. Only one of the 48 2D SDS-PAGE -purified NPC possessing an apparent M_r ~94,000 and pI ~6.5 gave a strong hybridization signal for HIV-1 *pol* (arrow) in Fraction R. **B:** Fraction M1. Row 1: a, M_r ~30,000, pI ~6.9; b, M_r ~28,000, pI ~8.1; c, M_r ~25,000, pI ~5.8; d, M_r ~17,000, pI ~8.5; e, M_r ~16,000, pI ~6.7; f, M_r ~15,000, pI ~8.6; g, M_r ~14,000, pI ~5.0; h, M_r ~42,000, pI ~5.3. Row 2: a, M_r ~29,000, pI ~7.0; b, *M_r ~31,000, pI ~8.7; c, M_r ~22,000, pI ~5.7; d, M_r ~16,000, pI ~8.3; e, M_r ~16,000, pI ~5.8; f, M_r ~14,000, pI ~8.6; g, M_r ~10,000, pI ~6.9; h, M_r ~43,000, pI ~5.8. Row 3: a, M_r ~30,000, pI ~6.0; b, M_r ~29,000, pI ~8.6; c, M_r ~22,000, pI ~5.3; d, M_r ~15,000, pI ~8.4; e, M_r ~15,000, pI ~5.8; f, M_r ~10,000, pI ~8.5; g, M_r ~10,000, pI ~6.9; h, M_r ~55,000, pI ~7.0. Row 4: a, M_r ~29,000, pI ~6.1; b, M_r ~25,000, pI ~7.6; c, M_r ~21,000, pI ~5.1; d, M_r ~14,000, pI ~8.0; e, M_r ~18,000, pI ~5.7; f, M_r ~25,000, pI ~5.4; g, M_r ~68,000, pI ~5.8; h, M_r ~68,000, pI ~7.0. Row 5: a, M_r ~29,000, pI ~5.2; b, M_r ~26,000, pI ~7.0; c, M_r ~22,000, pI ~8.0; d, M_r ~12,000, pI ~8.5; e, M_r ~17,000, pI ~5.5; f, M_r ~16,000, pI ~4.9; g, *M_r ~47,000, pI ~5.1; h, M_r ~60,000, pI ~8.1; Row 6: a, M_r ~29,000, pI ~5.7; b, M_r ~22,000, pI ~7.1; c, M_r ~19,000, pI ~8.5;d, M_r ~15,000, pI ~6.7; e, M_r ~20,000, pI ~5.5; f, M_r ~15,000, pI ~4.9; g, *M_r ~40,000, pI ~5.0; h, blank. Three of 48 2D SDS-PAGE-purified NPC of apparent M_r ~40,000, pI ~5.0; M_r ~47,000, pI ~5.1 and M_r ~31,000, pI ~8.7, respectively, were shown to strongly hybridize with HIV-1 *pol* from Fraction M2 (arrows). **C:** Fraction R. This depicts the same slot blot shown in **A** after it was stripped and rehybridized with HIV-1 *env* probe. Autoradiography was performed as in **A**.

β-casein gene, and the isolated lymphoma NPC were able to synthesize β-casein mRNA [Rosenberg-Nicolson and Nicolson, 1992a; Nicolson and Nicolson, 1993].

We also found that leukocyte NPC in their isolated form contained a variety of enzymatic activities that could be modulated by addition of low concentrations of particular cytokines. For example, we found that the NPC isolated from the leukocyte nuclei of patients with chronic myelogenous leukemia (CML) contained DNA polymerase activities that were sensitive to low concentrations of cytokines (~10 U) such as interferon-α (IFN-α) [Nicolson et al.,

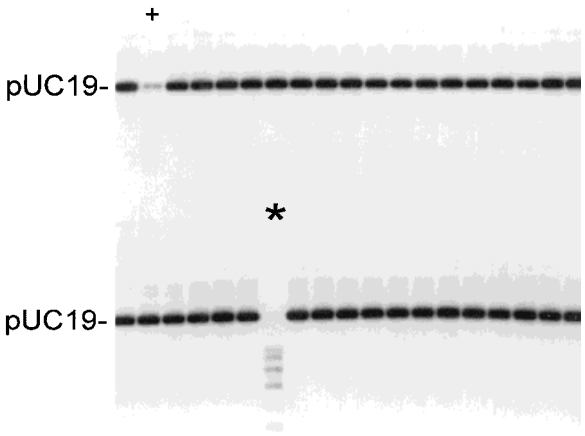

Fig. 4. Human immunodeficiency virus type 1 (HIV-1) *pol* gene-positive complex (NPC) possess varying degrees of in vitro endonuclease activity. This autoradiogram presents the results using linearized pUC19 plasma as substrate. The following HIV-1 *pol* gene-positive two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)-purified NPC were capable of degrading the pUC19 plasmid: M_r ~94,000 pI ~6.5, from fraction R (*), and M_r ~47,000 pI ~5.1 from fraction M2 (+). All other NPC were negative in this assay.

1995]. These observations led us to hypothesize that cytokines such as IFN-α might enter cells and be transported to the nucleus, where they might act directly on DNA polymerase complexes. In these studies, we were able to demonstrate that CML patients whose leukocyte nuclear NPC fractions contained DNA polymerase activity sensitive to in vitro inhibition by IFN-α were entirely from patients that were responsive to the in vivo administration of this cytokine. By contrast, patients whose leukocyte NPC fractions were insensitive to in vitro inhibition by IFN-α were unresponsive to the in vivo administration of IFN-α (Nicolson et al., 1995]. Thus, we were able for the first time to predict cytokine responsiveness in the clinical setting. We also found that that multiple NPC fractions from the leukocytes of CML patients with more progressive disease contained more c-*abl*, *p53*, and *bcl*-2 gene sequences than the same NPC fractions from CML patients with less advanced disease [Nicolson et al., 1996]. Since the NPC fractions from CML patients contained transcription- and proliferation-associated enzymes [Rosenberg-Nicolson and Nicolson, 1992a,b; Nicolson et al., 1995], the results suggested a possible role for certain tightly bound genes in the progression of CML. Further research will be necessary to confirm or not the notion that particular NPC may be involved in the progression of CML.

The NPGT technique has also been used to study infection of cells by microorganisms, such as *Mycoplasma* and other bacteria [Nicolson and Nicolson, 1996b; Nicolson et al., 1998a]. For example, using NPGT, we looked for the presence of mycoplasmal genes in leukocytes isolated from patients with Gulf War illnesses, chronic fatigue syndrome, fibromyalgia syndrome, and rheumatoid arthritis; we conducted similar experiments with forensic polymerase chain reaction (PCR) [Nicolson and Nicolson, 1996b; Nicolson et al., 1998b; Haier et al., 1999; Nasralla et al., 1999]. Using these two techniques, we were able to identify the presence of multiple mycoplasmal genes in the leukocytes of chronically ill patients at significantly higher frequencies (40–65%) than in control leukocytes from normal donors (0–9%). For example, we isolated NPC from the blood leukocyte nuclei from 170 patients with Gulf War illness; 77 (~45%) of these patients were positive for intracellular mycoplasmas. By contrast, we found only 2/41 (~5%) of nondeployed controls to be positive for this type of intracellular infection [Nicolson et al., 1998b]. Of the 77 patients who were positive for mycoplasmal infections, we found that most were infected with *Mycoplasma fermentans* (47/77, or 61% of the *Mycoplasma*-positive patients), whereas only a few were infected with *Mycoplasma genitalium* (<10% of the *Mycoplasma*-positive patients). Although not determined, we presume that many of the remaining *Mycoplasma*-positive patients had infections with *Mycoplasma pneumoniae* or other species. Although we did not determine the presence of additional species of mycoplasmas, we know from studies of subjects with chronic fatigue syndrome and fibromyalgia syndrome that many patients have multiple species of mycoplasmas in their blood, especially those who have been ill for several years [Nasralla et al., 1999].

In HIV-1-infected H9 cells, we found that various NPC contain loosely or tightly bound HIV-1 gene sequences. For example, we determined that four purified NPC components contained tightly bound HIV-1 *pol*. The reverse-transcribed HIV-1 genome possesses five *Msp*I restriction sites, one of which is ~417 bp downstream from the HIV-1 *pol* sequence. As the

HIV-1 *pol* gene does not contain a *Msp*I restriction site, the use of *Msp*I restriction to isolate the NPC should not have resulted in fragmentation of the *pol* gene. Therefore, these NPC are likely to contain *pol* gene sequences that were not cut by *Msp*I during the purification process. We also observed that two NPC that were isolated with tightly bound HIV-1 *pol* gene sequences contained endonuclease activity that was detected using an in vitro assay that measures the degradation of a standard plasmid substrate (pUC19). This result suggests the hypothesis that the association of the HIV-1 *pol* gene with NPC components that contain endonuclease activity might alter the host's endonuclease-associated cellular processes, such as programmed cell death or apoptosis. Possible alterations in cellular endonuclease activity due to interactions with HIV-1 genes might be important in initiating cell death or in some cases in stimulating cell proliferation. Although we do not have direct evidence for this notion, we cannot rule out that the presence of the HIV-1 *pol* gene in association with NPC that contain endonuclease activity may alter in some way this enzymatic activity, which may be important in apoptosis. That this is not an indirect effect of having the HIV-1 *pol* gene present was shown in experiments in which the HIV-1 gene sequences were added to isolated NPC; the added gene sequences had no effect on enzymatic activity, suggesting that the effect of the *pol* gene on enzymatic activity requires insertion or tight association with specific NPC (unpublished observations). The precise interactions and ensuing biochemical relationships between the HIV-1 *pol* and other HIV-1 genes, their respective gene products, and the host DNA polymerases and other proliferation- and apoptosis-related enzymes will require further investigation. Ultimately, it will be necessary to ascertain if and how a host's NPC functions are affected by HIV-1 gene-NPC associations.

Comments on NPC Structures and Interactions

We envision that the NPC and their constituents are composed of layers of compacted nucleic acid engulfing NPC chromatin regions. Thus, our experiments on HIV-1 infection of human H9 cells may be thought of as producing a pattern of specific DNA-NPC associations or chromatin NPC interactions. By contrast, studies that have focused only on HIV-1 integration in terms of primary DNA sequence did not discern any particular pattern of integration or association [Varmus, 1988]. From our data, we speculate that there may be specific macromolecular relationships between various NPC that contain specific gene sequences, such as the NPC in fractions M1 and M2 of HIV-1-iinfected H9 cells that contain *p53*, 18s rDNA, HIV-1 *pol*, *rev*, and *tat*. In addition, we speculate that specific macromolecular relationships may exist between the tightly bound HIV-1 *pol* and loosely bound HIV-1 *tat* genes in the NPC fraction R. However, considerable research will be necessary to begin to delineate any relationships between these complex macromolecular interactions and any functional nuclear activities.

Recent observations in our laboratories suggest that the relatively insoluble chromatin NPC in these studies contain components that may be subsets of well-characterized transcription factors. They most likely are also related to chromatin structures associated with nuclear matrix (or scaffolding) or chromatin loops, or both, but further experiments will be necessary to provide evidence for these notions.

A principal conclusion from our studies is that at least one aspect of HIV-1 infection may be characterized by a specific pattern of association of HIV-1 reverse-transcribed DNA, and specific NPC macromolecules that, at least in vitro, possess DNA endonuclease activity. Analyses of moieties of the HIV-1 genome and concomitant macromolecular association of HIV-1 genes in chromatin could provide a novel means to map viral integrations in eukaryotic nuclei and study chromatin macromolecular relationships between virus genes, host genes, and regulatory sequences and enzymes involved in critical cellular processes. Further studies will be important in understanding viral integration, association with NPC, and chromatin organization. Studying viral gene-containing NPC, their interactions, and macromolecular relationships may eventually influence the design of novel approaches to circumvent retroviral integration, replication, and cell death.

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