The in Vitro DNA-binding Properties of Purified Nuclear Lamin Proteins and Vimentin*

(Received for publication, September 5, 1989)

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The ability of purified nuclear lamin A, lamin B, lamin C, and vimentin from Ehrlich ascites tumor cells to bind nucleic acids was investigated in vitro via a quantitative filter binding assay. At low ionic strength, vimentin bound more nucleic acid than the nuclear lamins and showed a preference for G-containing nucleic acids. Nuclear lamins A and C were quite similar in their binding properties and bound G- and C-containing nucleic acids preferentially. The binding of poly(dT) by the lamins A and C was reduced in competition experiments by both poly(dG) and poly(dC), but not by poly(dA). Lamin B bound only oligo and poly(dG); no other nucleic acids tested were bound or could compete with the binding of oligo(dG), Vimentin, lamin A, and lamin C specifically bound a synthetic oligonucleotide human (vertebrate) telomere model. The $K_a$ for vimentin (2.7 x 10$^7$ M$^{-1}$) was approximately 10-fold higher than those for lamin A (2.8 x 10$^6$ M$^{-1}$) and lamin C (2.9 x 10$^6$ M$^{-1}$). Lamin B did not bind detectable amounts of the telomere model. The ability of purified nuclear lamins A and C, lamin C, and vimentin to bind with specificity to synthetic double-stranded DNA and poly(dT) among single-stranded homopolymers in competition assays (15). We have developed methods for large scale simultaneous purification of vimentin and lamins A, B, and C from Ehrlich ascites tumor cells (16) and describe here a further separation of lamins A and C by preparative gel electrophoresis. We therefore chose to employ a filter-binding assay to study the DNA-binding properties of the individual lamin proteins and vimentin. We report here that lamins A and C closely resemble vimentin in DNA-binding properties and that lamin B shows low binding activities with the DNAs employed. In addition, lamin A, lamin C, and vimentin, but not lamin B, bind with moderate affinity a synthetic oligonucleotide containing human (vertebrate) telomere repeat sequences.

EXPERIMENTAL PROCEDURES

Materials—Ehrlich ascites tumor cell vimentin and nuclear lamin proteins A, B, and C were prepared as previously described (16). Lamin A and C were separated from each other by preparative gel electrophoresis in 9–15% polyacrylamide gradient gels containing 6% acetic acid and 6 M urea. Lamin B was further purified by preparative SDS-gel electrophoresis on 9–15% polyacrylamide gradient gels (17). Lamin A and C were visualized by staining very briefly (~1 min) in Coomassie Blue (16) and lamin B was visualized by copper staining (18). Gel fragments containing the individual lamins were pulverized, and the proteins were eluted with a large volume of 6 M urea, 6 mM 2-mercaptoethanol (for lamins A and C) or several volumes of 0.1% SDS, 10 mM EDTA, pH 7.6, 6 mM 2-mercaptoethanol (for lamin B). Removal of SDS from lamin B was essentially as described (19). Batch adsorption on CM-Sepharose CL-6B was employed for lamins A and C and on DEAE-Sepharose CL-6B for lamin B. Elution with

1 After original submission of this manuscript, some of these results were presented in poster form at the 1989 ASCB Summer Research Conference Chromosome Structure and Segregation, September 8–12, 1989 at Airlie, Virginia.

2 The abbreviations used are: SDS, sodium dodecyl sulfate; Hum 1, double-stranded synthetic oligonucleotide containing authentic human (vertebrate) telomere repeats; SSD, E. coli single-stranded DNA-binding protein; GP92, bacteriophage T4 gene 32 protein.

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high salt and dialysis utilized the same buffers employed during earlier steps in the purification (16).

A self-complementary oligonucleotide (5'-CCCTAAAGGTTAGGG-3') was synthesized on a Beckman System I Plus DNA synthesizer and purified on 10% denaturing acrylamide gels. The conversion of this and a random sequence oligonucleotide (14) to double-stranded telomere models and analysis were described (14). Other oligo- and polynucleotides were purchased from Pharmacia-LKB (Freiburg, Federal Republic of Germany). Single-stranded DNAs were prepared by boiling double-stranded DNAs for 15 min then quickly cooling in an ice bath. The concentrations of all of the nucleic acids employed were measured spectrophotometrically, assuming 1 mg/ml = 20 μg. The size of the nucleic acids was confirmed and/or estimated by gel electrophoresis and comparison to standards derived from restriction digests of bacteriophage DNA or plasmids. Nucleic acids were 5'-end-labeled with 32P using 14 polynucleotide kinase (Pharmacia-LKB). Procedures for DNA manipulations were essentially as described (20). Other reagents were as described (14).

Methods—Standard reactions, as previously described for vimentin and nuclear lamins (14), contained 10 pmol of the respective protein monomers (i.e., 0.5 μg of vimentin, 0.7 μg of lamin A, 0.65 μg of lamin B, or 0.6 μg of lamin C) and a variable amount of nucleic acid (see "Results") in a total volume of 100 μl of 10 mM Tris-HCl, pH 7.4, 0.1 mM EDTA. Under these conditions, the intermediate filament proteins should be in the form of tetramers. The purified proteins were stored at -70 °C in small aliquots in 10 mM Tris-HCl, pH 7.4, 0.1 mM EDTA at a concentration of 1 μM protein. A fresh aliquot was used for each experiment to avoid the aggregate formation associated with multiple rounds of freeze-thawing. Competition experiments were performed with a constant amount of labeled nucleic acid (either 5 pmol of [32P]oligo(dG)12-18 or 5 pmol of [32P]-poly(dT)) and protein (2.5 pmol of tetramers of the indicated proteins) plus an equal amount of the unlabeled nucleic acids. The protein-nucleic acid interactions were measured by a quantitative filter-binding assay (14). To determine the salt dependence of the protein-nucleic acid interactions, the filter-bound complexes were washed three times each with 5 ml of 10 mM Tris-HCl, pH 7.4, 0.1 mM EDTA. Additionally, some incubations (see "Results") contained 150 mM KCl in addition to the other standard components. Results were corrected for nucleic acid bound to the filters in the absence of protein (usually 1-5% of input nucleic acid). The results are presented as picomoles of nucleic acid bound. The data represent averages of three or more separate experiments or typical results, as indicated. The maximum coefficient of variation (standard deviation + mean) was 24%; typically it was less than 10%. Scatchard analysis (22) was performed as described (14).

RESULTS

The binding of the individual nuclear lamins and vimentin to single-stranded nucleic acids was first performed at low ionic strength using end-labeled oligo(dN)12-18 (Fig. 1). These oligonucleotides were chosen due to their uniform size on gel analysis, although the oligo(dG)12-18 showed some dimer formation, presumably due to interstrand G·G base pairing (20). No difference was seen with oligo(dG)12-18 used before or after boiling to reduce secondary structure (results not shown). Data for oligo(dA)12-18 are not presented since none of the proteins bound detectable amounts of oligo(dA)12-18. All four proteins displayed a low affinity for oligo(dT)12-18. Vimentin bound more oligonucleotide with a higher affinity (in the order oligo(dG)12-18 > oligo(dC)12-18 > oligo(dG)12-18) than did the nuclear lamins. Lamin A and C showed the highest affinity for oligo(dG)12-18 (followed by oligo(dC)12-18 > oligo(dG)12-18). Lamin D bound oligo(dG)12-18 and oligo(dC)12-18 with similar affinities; both were bound much better than oligo(dT)12-18. As seen in Fig. 1 (note scale differences), lamin B and C were similar in their binding of oligo(dG)12-18 and oligo(dC)12-18. Lamin A bound about twice as much of these oligonucleotides as both lamin B and C.

The binding of these proteins to single-stranded nucleic acids was further investigated using poly(dN). The relative

![Fig. 1. Binding of oligo(dN)12-18 oligonucleotides by purified intermediate filament subunit proteins. The indicated amounts of [32P]-labeled oligonucleotides were incubated with the purified proteins (10 pmol of monomer) for 10 min at 25 °C in a total volume of 100 μl of 10 mM Tris-HCl, pH 7.4, 0.1 mM EDTA. The amount of oligonucleotide bound was determined by a filter binding assay as described under "Experimental Procedures." Oligonucleotides employed were: O, oligo(dC)12-18; X, oligo(dG)12-18; +, oligo(dT)12-18. Proteins tested were panel A, lamin A; panel B, lamin B; panel C, lamin C; panel D, vimentin. No binding of oligo(dA)12-18 was observed for any of these proteins.](http://www.jbc.org/)

| Table I
| Polynucleotide | Lamin A | Lamin B | Lamin C | Vimentin |
|----------------|---------|---------|---------|----------|
| Poly(dA)       | 0.04    | 0.0      | 0.05    | 0.02     |
| Poly(dT)       | 1.0     | 0.9      | 0.8     | 0.1      |
| Poly(dG,dC)    | 0.4     | 0.1      | 0.4     | 0.7      |
| Poly(dG-dC)    | 0.2     | 0.1      | 0.4     | 0.7      |
| Poly(dA)       | 0.04    | 0.03     | 0.05    | 0.3      |
| Poly(dA-dT)    | 0.03    | 0.01     | 0.01    | 0.09     |
| Poly(dG)       | 0.03    | 0.02     | 0.03    | 0.2      |
| Poly(dG-dC)    | 0.01    | 0.01     | 0.01    | 0.04     |

The binding of the individual nuclear lamin proteins and vimentin to single-stranded nucleic acids was first performed at low ionic strength using end-labeled oligo(dN)12-18 (Fig. 1). These oligonucleotides were chosen due to their uniform size on gel analysis, although the oligo(dG)12-18 showed some dimer formation, presumably due to interstrand G·G base pairing (20). No difference was seen with oligo(dG)12-18 used before or after boiling to reduce secondary structure (results not shown). Data for oligo(dA)12-18 are not presented since none of the proteins bound detectable amounts of oligo(dA)12-18. All four proteins displayed a low affinity for oligo(dT)12-18. Vimentin bound more oligonucleotide with a higher affinity (in the order oligo(dG)12-18 > oligo(dC)12-18 > oligo(dG)12-18) than did the nuclear lamins. Lamin A and C showed the highest affinity for oligo(dG)12-18 (followed by oligo(dC)12-18 > oligo(dG)12-18). Lamin D bound oligo(dG)12-18 and oligo(dC)12-18 with similar affinities; both were bound much better than oligo(dT)12-18. As seen in Fig. 1 (note scale differences), lamin B and C were similar in their binding of oligo(dG)12-18 and oligo(dC)12-18. Lamin A bound about twice as much of these oligonucleotides as both lamin B and C.

The binding of these proteins to single-stranded nucleic acids was further investigated using poly(dN). The relative

mobilities of poly(dA), poly(dC), and poly(dT) in urea/acrylamide gels were similar and suggested a length of approximately 250 bases, in agreement with the manufacturer's data. The poly(dG), on the other hand, migrated as if it were approximately 10 times smaller, suggesting a shorter average chain length and/or anomalous electrophoretic behavior. It was not possible to 5'-end label the poly(dC) preparation to a specific activity higher than 250 cpm/pmol, a value too low to use in the filter-binding assay. Data for the binding of the polynucleotides are presented in Table I. As seen for the oligo(dA)12-18, none of the proteins bound significant amounts of poly(dA). The relative binding of poly(dG) by all four proteins was essentially similar. On a molar basis, the nuclear lamins A and B bound more poly(dG) than poly(dT), whereas vimentin and lamin C bound similar amounts of poly(dG) and poly(dT). The relative binding of poly(dT) by these proteins was found to be vimentin > lamin A ≈ lamin C > lamin B. Heat denaturation of the [32P]-poly(dG) had no effect on the binding observed (data not shown).

Competition experiments with these single-stranded oligo- and polynucleotides were performed to avoid problems asso-
associated with the labeling of the nucleic acids and to permit direct comparisons of binding affinities (Table II). For both the oligo- and polynucleotides, oligo- and poly(dG) were found to be the best competitors for oligo(dG) and poly(dT) binding by all of the proteins. Neither oligo(dA)₁₂-₁₈, oligo(dC)₁₂-₁₈, nor oligo(dT)₁₂-₁₈ had an effect on the binding of poly(dG)₁₂-₁₈ by all four proteins. Neither oligo(dA), oligo(dC)₁₂-₁₈, nor poly(dT) had no effect on the binding of poly(dT) by vimentin or lamin B (probably due to the negligible binding of poly(dT) by lamin B). Likewise, poly(dA) did not compete with labeled poly(dT) for binding by all four proteins, whereas, as expected, unlabeled poly(dT) did compete (Table II). These results suggest that the affinity of all four proteins is higher for oligo- and poly(dG) than oligo- or poly(dT), which, in turn, is bound with higher affinity than poly(dA). These competition assays also failed to clearly demonstrate a preferential nucleotide binding other than the binding of oligo(dG)₁₂-₁₈ by lamin B, consistent with the low binding of all the other nucleic acids seen in the direct binding assays.

A variety of single-stranded and double-stranded random and copolymers were also employed as substrates for binding (Table I). Lamin B failed to bind any of these nucleic acids, save for a low binding to poly(dA,dT) and poly(dG,dC). Lamin A and C bound single stranded poly(dA,dT) equally well but failed to bind either double-stranded poly(dA)-(dT) or poly(dA-dT)-(dA-dT). Vimentin also bound well to single-stranded poly(dA,dT) and not at all to double-stranded poly(dA-dT)-(dA-dT) but, in contrast to the lamins A and C, vimentin also bound double-stranded poly(dA)-(dT) albeit at a lower level than single-stranded poly(dA,dT). Lamins A and C and vimentin bound single-stranded poly(dG,dC) about as well as poly(dA,dT). Double-stranded poly(dG)-(dC) was bound only by vimentin, and poly(dG,dC), was not bound by any of the proteins. These results suggest that a minimum number or arrangement of G residues that might occur in the random copolymer is required for binding and that conformation of the nucleic acids might also play a role in binding (i.e. loops or hairpins that might form from the random copolymers).

The ability of the lamins and vimentin to bind a synthetic double-stranded oligonucleotide telomere model with a single-stranded overhang (containing the human telomere repeat TTAGGG (Ref. 24) and hereafter referred to as Hum 1) was investigated using the filter-binding assay. Lamin A, lamin C, and vimentin bound detectable amounts of the Hum 1 oligonucleotide (Fig. 2, Tables III and IV). Scatchard analysis showed that vimentin bound more of the Hum 1 oligonucleotide (on a molar basis) with a 10-fold higher affinity than lamins A and C (Table IV). As previously reported for vimentin (14), lamins A and C bound about three times more of the oligonucleotide than a similar oligonucleotide with a random sequence (Table III). Lamin B showed little or no affinity for either of the synthetic oligonucleotide telomere models under the conditions employed (Tables III & IV), although it should be mentioned that a Kᵦ below approximately 1 x 10⁶ M⁻¹ would not be detected in this system.

All of the preceding data were obtained under conditions of

![FIG. 2. Binding of a synthetic oligonucleotide containing authentic human telomere repeat sequences (Hum 1; see Table III) by lamin A (●), lamin B (○), lamin C (×), and vimentin (■). Each protein (2.5 pmol of tetramers) was incubated for 10 min at 25 °C with the indicated amounts of ³²P-labeled oligonucleotide in a total volume of 100 μl of 10 mM Tris-HCl, pH 7.4, 0.1 mM EDTA. The amount of oligonucleotide bound was determined by a filter binding assay as described under “Experimental Procedures.” These are typical results. Additional data derived by Scatchard analysis are presented in Table IV.](http://www.jbc.org/)

**TABLE II**

| Protein | Nucleic acid added | ³²P-Labeled nucleic acid bound pmol |
|---------|--------------------|-----------------------------------|
| Lamin A | Oligo(dG)₁₂-₁₈     | 1.2 |
|         | Oligo(dA)₁₂-₁₈     | 1.3 |
|         | Oligo(dC)₁₂-₁₈     | 0.5 |
|         | Oligo(dT)₁₂-₁₈     | 0.4 |
|         | Poly(dT)           | 0.7 |
|         | Poly(dA)           | 0.1 |
|         | Poly(dC)           | 0.05 |
|         | Poly(dG)           | 0.1 |
|         | Poly(dT)           | 0.1 |
| Lamin B | Oligo(dG)₁₂-₁₈     | 1.1 |
|         | Oligo(dA)₁₂-₁₈     | 1.3 |
|         | Oligo(dC)₁₂-₁₈     | 1.1 |
|         | Oligo(dG)₁₂-₁₈     | 0.4 |
|         | Oligo(dT)₁₂-₁₈     | 0.9 |
|         | Poly(dT)           | 0.1 |
|         | Poly(dA)           | 0.3 |
|         | Poly(dC)           | 0.1 |
|         | Poly(dG)           | 0.1 |
|         | Poly(dT)           | 0.1 |
| Lamin C | Oligo(dG)₁₂-₁₈     | 1.1 |
|         | Oligo(dA)₁₂-₁₈     | 1.1 |
|         | Oligo(dC)₁₂-₁₈     | 1.2 |
|         | Oligo(dG)₁₂-₁₈     | 0.5 |
|         | Oligo(dT)₁₂-₁₈     | 1.1 |
|         | Poly(dT)           | 0.4 |
|         | Poly(dA)           | 0.2 |
|         | Poly(dC)           | 0.0 |
|         | Poly(dG)           | 0.1 |
|         | Poly(dT)           | 0.1 |
| Vimentin| Oligo(dG)₁₂-₁₈     | 1.8 |
|         | Oligo(dA)₁₂-₁₈     | 2.1 |
|         | Oligo(dC)₁₂-₁₈     | 2.0 |
|         | Oligo(dG)₁₂-₁₈     | 0.8 |
|         | Oligo(dT)₁₂-₁₈     | 1.9 |
|         | Poly(dT)           | 1.7 |
|         | Poly(dA)           | 2.1 |
|         | Poly(dC)           | 1.9 |
|         | Poly(dG)           | 0.5 |
|         | Poly(dT)           | 1.2 |
DNA Binding by Nuclear Lamin Proteins and Vimentin in Vitro

The HaeIII restriction site employed for analysis of oligonucleotide structure is overlined on the upper strand. Binding experiments were performed under standard conditions as described under “Experimental Procedures.” Each value is the average amount of oligonucleotide bound in four experiments when 5 pmol of the indicated 32P-labeled oligonucleotide (specific activity = 10,000 cpm/pmol) was added to 2.5 pmol of tetramers of the indicated proteins.

**DISCUSSION**

The quantitative binding assays performed at low ionic strength with the oligonucleotide homopolymers (12-18 nucleotides long) clearly demonstrate that vimentin binds more nucleic acid with higher affinity than the lamins, showing a clear preference for oligo(dG)12-18, and that the purified lamins A and C bind both oligo(dG)12-18 and oligo(dC)12-18 better than oligo(dT)12-18. Lamin A bound reproducibly more oligo(dG)12-18 than lamin C. None of the four proteins bound oligo(dA)12-18. Similar results were seen with the polynucleotides. The competition assays showed oligo- and poly(dG) to be the best competitors for binding of both oligo(dG)12-18 and poly(dT) for all proteins tested. The results obtained with these single-stranded nucleic acids for vimentin are largely in agreement with previous results obtained in competition experiments performed on a mass (microgram) basis using sucrose gradient analysis of the reaction products (9). Our present results suggest that lamin B has a low affinity for the nucleic acids tested (except for poly(dG), Table I), whereas lamins A and C each bind dG-containing much better than dT-containing single-stranded nucleic acids. This is in direct contrast to studies performed with a mixture of all three lamins (nuclear matrix proteins), where poly(dT) was found to be bound best (15).

All of the various homo- and copolymer, single-stranded DNA molecules employed in this study were bound better than double-stranded molecules. Vimentin generally bound more of these oligo- and polynucleotides in comparison to the nuclear lamins. Lamins A and C were generally similar in their binding, except for a reduced binding of single-stranded poly(dG,dC) by lamin A. The results presented here for vimentin are in good agreement with those obtained previously, except for double-stranded poly(dA,dT), which in the present studies was bound better than previously found in competition experiments (9) using a different analytical method. We show that lamins A and C bind single-stranded poly(dA,dT) and poly(dG,dC) whereas double-stranded poly(dA) (dT), poly(dA dT) (dA dT), poly(dG) (dC), or poly(dG-dC)- (dG-dC) were essentially not bound at all. Previous studies found that nuclear matrix proteins (essentially a mixture of all three lamins) preferentially bound AT-rich double-stranded DNA (15).

With synthetic copolymers (i.e. poly(dA,dT) and poly(dG,dC)), there was no marked preference for a specific base composition (since both are bound), suggesting that hairpins or other structural elements may be involved in the interactions observed. These conclusions are at odds with those previously drawn from competition assays employing a mixture of nuclear lamins and various nucleic acid substrates (15) or assays from our laboratory with purified vimentin (9). No attempts were made in these previous studies to correct for the molecular weight of the nucleic acids employed. Our experience in performing the experiments described in this paper suggest this may be a significant source of error in comparing results and may explain some of the differences and discordances noted here. Additionally, previous experiments by Comings and Wallack (15) were done on a microgram basis with the nuclear matrix proteins present in a molar excess relative to the nucleic acids tested. They required more than 5 µg of unlabeled mouse DNA to compete to 50% of control binding levels observed with 1 µg of 3H-labeled mouse DNA (15). We took care to always offer the nucleic acids in molar excess (assuming the intermediate filament proteins to be present as tetramers) and our competition assays showed that, as expected, 5 pmol of unlabeled oligo(dG) or poly(dT) reduced the binding observed with 5 pmol of 3P-labeled oligo(dG) or poly(dT), respectively, to about 50% of control levels (Table II).

Vimentin and lamins A and C were found to be generally
quite similar in their general nucleic acid-binding characteristics. This result is perhaps expected since the deduced amino acid sequences of these proteins are highly homologous (4, 5). However, this homology is largely restricted to the central, α-helical rod domain; the N and C termini of vimentin show no overall homology to those of lamins A and C. The non-α-helical N-terminal domain of vimentin has been shown to be the site of nucleic acid binding (14). Lamins A and C are translated from differentially processed mRNAs derived from a single transcript. They are identical for the first 566 amino acid residues. Lamin C has an additional six amino acids, while lamin A has an additional 98 amino acids at its C terminus (4, 5). Thus, it is no surprise that lamins A and C show similar nucleic acid binding properties; any differences, such as that seen in binding oligo(dC)12-18, perhaps reflect differences present in the C termini of these proteins. The primary sequence of mouse lamin B, deduced from the nucleotide sequence of a cDNA, has been determined (6). The homology of lamin B to lamin A and C (and other intermediate filament proteins) is only 68% and is restricted to the central rod domain. The non-α-helical N- and C-terminal regions of lamin B share no extensive regions of homology with other known intermediate filament proteins including lamins A and C (although the functional sequence for nuclear localization is present in the C terminus). The short (35 residues) N-terminal head region of lamin B has a net positive charge that may be responsible for the low level binding of G-containing nucleic acid by lamin B. It remains to be shown what domain(s) of the lamin proteins is involved in nucleic acid binding, as has been done for other intermediate filament proteins. The binding site for nucleic acid (known to be in the N terminus of vimentin (14)) may be large enough to simultaneously bind several small oligonucleotides, since on a molar basis more oligo(dG)12-18 than poly(dG) was bound by vimentin and lamin A (Fig. 1, Table I and data not shown).

Our previous study (14) with vimentin showed that this protein is capable of specifically binding to a short oligonucleotide containing several telomere repeat sequences (i.e. TTAGG, TTAG, and TGGT) with a $K_a \approx 4 \times 10^4$ M⁻¹. In this previous study, we described a qualitative interaction of lamins A, B, and C with these model telomere oligonucleotides. In the present study, we have found that vimentin, lamin A, and lamin C bind another model telomere oligonucleotide (Hum 1) containing human telomere repeat sequences (TTAGGG). (This sequence, first described for human telomeres (24), exists in all mammalian telomeres, including mouse.) The $K_a$ for vimentin for this oligonucleotide ($K_a = 2.7 \times 10^5$ M⁻¹) is similar to that previously found for oligonucleotides containing telomere repeats of other organisms. The $K_a$ values of lamin A and lamin C (2.8 $\times 10^5$ M⁻¹ and 2.9 $\times 10^5$ M⁻¹) are 10-fold lower than those of vimentin. Lamins A and C bound about three times more of the Hum 1 oligonucleotide than one containing a random sequence. Values for lamin B were at or below the detection limits of the assay. Thus, vimentin, lamin A, and lamin C possess the ability to bind in vitro not only oligo- and polynucleotides with a preferred base composition but also to specifically bind a synthetic telomere model with moderate affinity. This extends observations made previously (25) that HeLa cell nuclear lamin proteins can bind (uncharacterized) HeLa DNA fragments in a gel-overlay assay. Presumably the gel-overlay assay permits the detection of lower affinity complexes than does the filter-binding assay described here, offering an explanation for the apparent discrepancy between the present and our previous results on the ability of lamin B to bind to telomere model oligonucleotides.

All of the foregoing data were obtained under conditions of low ionic strength. When preformed protein-nucleic acid complexes were washed with buffers containing 150 mM KCl, some but not all of the nucleic acid was eluted. These results are quantitatively identical to those obtained previously for vimentin (14) and suggest that the binding observed at low ionic strength may be due to both nonspecific, salt-liable, electrostatic interactions and more specific, salt-stable interactions. Similar differences in relative binding of nucleic acids at different ionic strengths have been described for hormone receptors (26, 27) and prokaryotic single-stranded DNA-binding proteins (28–30). The primary reason for doing the majority of our experiments at low ionic strength was to avoid the confounding effect of filament or paracrystalline array formation on the number of available binding sites. For vimentin, it has been previously shown that the nucleic acid-binding site resides in the non-α-helical N-terminal domain (14 and references therein) and that these sites are masked along the long axis of filaments due to their involvement in protein-protein interactions (31, 32). However, it has been shown by electron microscopy that the physical ends of vimentin filaments, presumably through their free N termini, are capable of interacting with DNA in vitro (13). Although the binding site for nucleic acids of the nuclear lamin proteins has not been localized, it is not unreasonable to predict that it may also be at least partially masked within the higher order structure of the nuclear lamins. In interphase cells, essentially all of the vimentin and nuclear lamin subunit proteins are organized into filaments. Thus, if these proteins actually bind to DNA in vivo, it is likely that they do so at the ends of filaments, at regions where filaments or nuclear lamina structure is disrupted or modified and/or after release of (modified) subunit proteins.

A number of interesting similarities and differences are noted when our binding data for these proteins are compared with those of other known DNA-binding proteins. Single-stranded DNA-binding proteins, such as Escherichia coli SSB and bacteriophage T4 GP32, show considerable structural homology to each other and have been characterized as binding to single-stranded DNA cooperatively and with no sequence specificity (33). The apparent association constants of SSB range from 1.5 $\times 10^6$ M⁻¹ for p(dT)₉ and 3 $\times 10^7$ M⁻¹ for p(dT)₄₉, up to 4.4 $\times 10^8$ M⁻¹ for high molecular weight single-stranded DNA, showing a clear cooperative effect with increasing template size (28). More rigorous studies with GP32 show net binding affinities to range from 10⁶ to 10¹⁰ M⁻¹, while the cooperativity effect due to protein-protein interactions during binding was ~10³; thus the actual $K_a$ values for this protein for polynucleotides ranged from 10⁶ to 10¹⁰ M⁻¹. At the other end of the spectrum of binding affinities are those proteins that specifically recognize a unique DNA sequence, such as steroid hormone receptors (26, 27) or the heat shock activator protein (34). These proteins have $K_a$ values of 10⁻⁶ to 10⁻¹⁰ M⁻¹ for their specific target sequences and display much lower affinities (10⁻⁶ M⁻¹) for other, nonspecific sequences. Many of the enzymes involved in DNA repair have $K_a$ values for DNA binding that fall between the two extremes already mentioned and thus can be considered to have "moderate" affinity for DNA. For example, E. coli photolyase, an enzyme that catalyzes the light-dependent repair of pyrimidine dimers, binds UV-irradiated plasmid DNA with a $K_a \approx 5 \times 10^5$ M⁻¹ (35). The $K_a$ values for the binding of Hum 1 by vimentin, lamin A, and lamin C are thus at the upper end of the range of $K_a$ values for the nonspecific, single-stranded DNA-binding proteins SSB and GP32 and are much lower than those of the sequence-specific hormone
receptor or heat shock activator proteins. They are within the same order of magnitude as that of *E. coli* photolyase, SSB, GP32, and the hormone receptors all display a reduced binding of nucleic acids with increasing ionic strength that is qualitatively similar to that reported here for lamins A and C and previously described for vimentin (14).

Previous qualitative studies from our laboratory (13) suggested that vimentin, like SSB and GP32, can interact cooperatively with superhelical plasmid DNA (but not linear or relaxed DNA) under conditions of low ionic strength similar to those employed here. It is not known whether the nuclear lamin proteins can also interact cooperatively with nucleic acids. We are currently constructing plasmids containing multiple copies of various telomere repeat sequences to address the question of target sequence size and cooperativity in binding. Although many of their nucleic acid-binding properties are quite similar to SSB and GP32, it is noteworthy that lamins A and C and vimentin possess moderate affinities for double-stranded oligonucleotide telomere models.

Another group of proteins has been described (36, 37) that bind specifically and tenaciously to telomeres of ciliates of protozoans. Two proteins of 55 and 43 kDa (as well as a 26-kDa degradation product of the 43-kDa species) form a telomeric complex containing the 3'-terminal telomeric repeat. These proteins remain bound even in the presence of 2 M NaCl but can be displaced by chaotropic reagents or elevated temperatures. A preliminary report (38) suggests that telomeric proteins of 55 and 68 kDa from the ciliate *Euplotes* are immunologically related to intermediate filaments and lamins and can polymerize into or associate with 10 nm fibres. Although murine vimentin, lamin A, and lamin C can bind model telomere oligonucleotides and show reduced binding of a random sequence oligonucleotide, it is quite clear that they differ considerably in their salt stability from the telomere-binding proteins characterized in ciliated protozoans.

A potential problem inherent in all of these studies is the size heterogeneity within and between the various nucleic acids. It is not unreasonable to assume that the base composition, conformation (single-stranded versus double-stranded, hairpins, etc.) and size (single or multiple targets for binding) of a nucleic acid are all equally important parameters for binding by these proteins. Our present study has tried to separate and/or minimize variations in these parameters by employing various sizes and types of synthetic nucleic acids. Likewise, the aggregation state of the proteins employed might play a role in modulating binding affinities and the number of available binding sites. Comings and Wallack (15), in their previous study using a crude mixture of nuclear matrix proteins, found it necessary to use freshly prepared material to minimize aggregate formation. We choose to separate and purify each of the nuclear lamin proteins to investigate the DNA-binding properties of the individual proteins and to reduce the tendency to form higher order aggregates or parasubunits (reviewed in Ref. 39 and references therein). Our data suggest that even our purified individual proteins exist in some higher order aggregate since, on a molar basis, tetramers of the nuclear lamin proteins bind substoichiometric quantities of the Hum 1 oligonucleotide (a double-stranded 60-mer with 3'-single-stranded overhangs). Our nuclear lamin proteins were isolated from proliferating cells and therefore are probably heterogeneous with respect to post-translational modifications that are cell cycle-associated, such as phosphorylation. This may also be partially responsible for the substoichiometric binding observed, if such modifications affect the ability of the nuclear lamins to bind nucleic acids. Another possibility is that our proteins were inactivated or denatured during purification in the presence of denaturing reagents. Since other intermediate filament proteins treated in the same manner are fully competent to assemble into intermediate filaments (see, for example, (19)), we believe our nuclear lamin proteins exist in largely native configurations. Another preparation of lamin B, obtained in low yields without SDS, was identical to the lamin B employed here in binding of polynucleotides (data not shown). Additionally, the procedure used by Comings and Wallack (16) for isolation of nuclear matrix proteins employed strongly denaturing conditions (freeze-thawing in buffered 2 M NaCl and 5 M urea or 1 M guanidine thiocyanate) and their preparations rapidly lost DNA-binding activity after removal (by dialysis) of the chaotropic reagent.

Our data are consistent with current concepts of the structure/function relationships of the nuclear lamin proteins. Lamin R exhibits low affinity binding of nucleic acids and appears to be responsible for anchoring the nuclear lamina to the nuclear membrane (3). Lamins A and C are thought to participate in the anchoring of chromatin to the nuclear periphery in interphase nuclei (5). Our results suggest that lamina A and C may also directly bind to specific, G-rich DNA sequences and perhaps contribute to the selection of sites to be anchored on the nuclear lamina. Even though their affinities for the Hum 1 oligonucleotide are only ~3 x 10^6 M^-1, it is conceivable that significant high specificity and high affinity binding may take place as a result of cooperative effects that might arise from interactions of many nuclear lamin molecules, organized into repeats along the meshwork of the nuclear lamina, with a sufficiently large DNA target sequence. The generation of high specificity of effect through low specificity binding of proteins to DNA has recently been proposed by Zucker and Villet (40). It should be emphasized that, although the nuclear lamin proteins are major components of the nuclear matrix, it is quite likely one or many other DNA-binding proteins are also involved in anchoring chromatin and modulating transcription and replication of DNA. We are currently investigating the interaction of these proteins with nucleic acids in vivo. Cress and Kurath (41) reported that vimentin is firmly (probably covalently) bound to single-stranded DNA in Chinese hamster ovary cells and is a protein candidate for the attachment of DNA within the nucleus. It will be of interest to see whether telomere sequences or other G-rich sequences are indeed bound by vimentin and nuclear lamins in vivo, as might be expected from their in vitro binding propensities.

**Acknowledgments**—We thank Sigrid Pfundstein and Annemarie Scherbarth for excellent technical assistance, Dr. Egon de Groot and Brigitte Seib for supplying the crude human telomere oligonucleotide, Christel Fabricius for doing the illustrations, and Heidi Klempp for secretarial work.

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