

## The Binding of Fluorochromes and Proteins to Cellulose-immobilized Nucleic Acids

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Columns containing DNA immobilized to cellulose were used to determine the quantity of fluorochromes and proteins bound to nucleic acids. The amount of dye bound to the DNA was directly proportional to the AT base content of the polymer. Of the three acridine derivatives tested, acranil had the highest, quinacrine mustard the lowest, and quinacrine intermediate affinity for the nucleic acids. The affinity of the dye Hoechst 33258 varied in relation to the AT base content of the polymer. The higher the AT base content of the DNA, the more poly-L-lysine and the less poly-L-arginine was bound. Binding of polyamino acids or histones to the cellulose-immobilized DNAs partly or wholly prevented the subsequent binding of dyes. Poly-L-lysine was more effective than poly-L-arginine in blocking the dye-binding to DNA. Complete blocking was obtained with protamine. Of the three histone fractions tested, the lysine-rich fraction was twice as effective in blocking dye binding as the intermediate fraction or the arginine-rich fraction.

The past few years have seen many investigations attempting to explain the reactions between fluorochromes and nucleic acid polymers. It has become clear that the reactions between acridine dyes and nucleic acids are base specific. Polyadenylic acid or polynucleotide mixtures with high adenine to guanine ratios increase the fluorescence intensity of the acridines in solution, whereas quenching occurs with polyguanylic acid or polynucleotide mixtures with low adenine to guanine ratios.<sup>1-5</sup> The fluorochrome Hoechst 33258 is an exception to this rule, since both polyadenylic acid and polyguanylic acid enhance its fluorescence intensity in solution. However, the increase in the fluorescence intensity of this dye is higher in mixtures

with polyadenylic acid than in mixtures with polyguanylic acid, and hence it, too, is base specific.<sup>6</sup>

The binding affinity of dyes to polymers can be studied in different ways, ultrafiltration, equilibrium dialysis and absorption or fluorescence titration being the methods most commonly used. Such studies have yielded inconclusive or conflicting results (*cf.* Refs. 7—12).

The use of immobilized nucleic acids has become wide spread in recent years, especially in the study of nucleic acid enzymes.<sup>13</sup> The application of dyes to immobilized nucleic acids is a suitable technique for dye-binding studies and in this paper we present results from such experiments. Furthermore the successive application of proteins and dyes to cellulose-immobilized nucleic acids allows a quantitation of the blocking effect of proteins on the binding of dyes to nucleic acids. Our data show that the affinity of fluorochromes as well as the binding of certain proteins to nucleic acids are dependent on the base content of the DNA.

### MATERIALS AND METHODS

*Nucleic acid derivatives.* The potassium salt of polyadenylic acid, the sodium salt of polyguanylic acid and the heteropolymerized nucleotide duplex polyadenylic-thymidylic acid were purchased from the Miles Laboratories, U.S.A., and the potassium salt of polycytidylic acid and the potassium salt of polyuridylic acid from Sigma Chemical, Co., U.S.A. DNA from *Clostridium perfringens*, from *Escherichia coli* and from *Micrococcus lysodeikticus* were purchased from Sigma Chemical, Co., U.S.A. DNA from calf thymus or from mouse liver was

extracted according to the method of Marmur.<sup>14</sup>

**Proteins.** The polyamino acids poly-L-arginine hydrochloride (mol.wt. 65 000) and poly-L-lysine hydrobromide (mol.wt. 180 000) and the calf thymus histone fractions "Type IIA" (containing all histones), "Type III" (lysine-rich fraction) and "Type IV" (arginine-rich fraction) as well as protamine and protamine sulfate were purchased from Sigma Chemical Co., U.S.A.

**Dyes.** Acranil and quinacrine were purchased from Bayer Leverkusen, W. Germany and quinacrine mustard from Polysciences, Inc., U.S.A. The dye Hoechst 33258 was a gift from Dr. H. Loewe, Hoechst Ag., Frankfurt, W. Germany.

**Labelling of DNA.** Mouse L-cells were labelled with <sup>3</sup>H-thymidine as described earlier.<sup>15</sup> The L-cell DNA was isolated according to the method of Marmur.<sup>14</sup> The specific activity of the L-cell DNA was approximately 165 counts/min per  $\mu$ g. The radioactivity was assayed by liquid scintillation in 10 ml of Insta-Gel solution from Packard Co., U.S.A.

**Preparation of nucleic acid cellulose.** Cellulose powder C F 11 was obtained from Whatman, England, and the DNA-cellulose was prepared using irradiation with ultraviolet light according to the method of Litman.<sup>16</sup>

The amount of DNA bound to cellulose was determined as follows: A known amount of nucleic acid cellulose (approximately 100 mg of dry DNA-cellulose) was boiled for 10 min in 5 ml of 5 % perchloric acid. The nucleic acid released into the supernatant was determined spectrophotometrically at 260 nm or by liquid scintillation counting. This method completely removed the DNA from the cellulose (cf. Table 1).

The nucleic acid cellulose columns were prepared as follows: The dry DNA-cellulose was suspended in 5 mM NaCl (pH 7.0) and the

DNA-cellulose allowed to settle during passage of several milliliters of 5 mM NaCl through the columns (1 cm in length, 2 cm in diameter). An excess of fluorochrome or protein (2 mg/ml in 5 mM NaCl, pH 7.0) was applied to the cellulose columns, and the columns were then washed extensively with 5 mM NaCl to remove unbound fluorochrome or protein. The fluorochrome or protein bound to cellulose-immobilized DNA was removed with 1 M HCl. The recovery of the dye or protein was complete, but no DNA was removed (cf. Table 1). The column effluent was collected in 2.5 ml fractions and the amount of fluorochrome or protein in the 5 mM NaCl fractions as well as in the fractions containing 1 M HCl was estimated. The acridine derivatives or poly-L-lysine did not bind to DNA-free acid-washed cellulose, whereas Hoechst 33258 and poly-L-arginine were bound. The values given for Hoechst 33258 or for poly-L-arginine are therefore corrected for this background of binding to cellulose alone.

**Protein assay.** L-Arginine and poly-L-arginine were assayed using the Sakaguchi dye reaction.<sup>17</sup> Poly-L-lysine and mixtures of poly-L-arginine plus poly-L-lysine were assayed by precipitation with trichloroacetic acid and by spectrophotometric estimation of the precipitate at 400 nm according to Bonner *et al.*<sup>18</sup> The amino acids and histones were determined by the method described by Lowry *et al.*<sup>19</sup>

**Dye assay.** The molar extinction coefficients of the fluorochromes at respective absorption maxima were: acranil,  $8.3 \times 10^8$  at 424 nm; quinacrine,  $8.5 \times 10^8$  at 424 nm; quinacrine mustard,  $14.6 \times 10^8$  at 424 nm; Hoechst 33258,  $32.7 \times 10^8$  at 345 nm.

#### Abbreviations.

poly(rA), polyadenylic acid  
poly(rC), polycytidylic acid  
poly(rG), polyguanylic acid  
poly(rU), polyuridylic acid  
poly(dA-dT), polyadenylic-thymidylic acid  
Q, quinacrine  
QM, quinacrine mustard

Table 1. The stability of cellulose-immobilized DNA.

Solution	Released DNA %
Distilled water	0.3
1 M HCl (pH 1.0)	0.5
2 M NaCl (pH 7.0)	1.0
0.25 M $\text{KH}_2\text{PO}_4$ —	
0.25 M $\text{K}_2\text{HPO}_4$ (pH 6.8)	0.5
0.1 M citric acid—	
0.2 M $\text{Na}_2\text{HPO}_4$ (pH 7.0)	0.4
1 M NaOH (pH 13.0)	40.6
Methanol	14.7
Ethanol (50 %)	13.4
Ethanol (100 %)	9.8
Perchloric acid (5 %, boiling for 10 min)	100.0

## RESULTS

The stability and availability of cellulose-immobilized DNA

To check the stability of the binding between native DNA and cellulose the release of isotopically labelled DNA was estimated after elution of DNA from the cellulose with various solutions (Table 1). It is shown that water or salt solutions at neutral pH-values did not remove DNA from the cellulose. However, at high pH values as much as approximately

40 % of the immobilized DNA is removed (1 M NaOH). Different alcohols removed approximately 10 % of the DNA. If the DNA-cellulose is boiled for 10 min in 5 % perchloric acid the DNA is completely removed.

The capacity of cellulose-immobilized DNA to react with nucleic acids passing through the column was tested as follows: Columns containing a cellulose-immobilized polynucleotide were perfused with complementary and non-complementary polynucleotides and the binding was measured. One mg of cellulose-immobilized poly(rA) bound 0.1 mg of poly(rU) but no poly(rA), poly(rC) or poly(rG). Correspondingly, 1 mg of cellulose-immobilized poly(rG) bound 0.16 mg of poly(rC) but no poly(rA) or poly(rU). These findings indicate that the cellulose-immobilized homopolymers retain their specificity of hybridization to complementary bases, but the binding capacity is not of the same magnitude as it is in solution. This is probably because the bonds between the cellulose and the polynucleotide compete with the pairing of complementary bases.<sup>13</sup>

In contrast, when a mixture of poly(rC) and poly(rG) was applied to poly (dA-dT)-cellulose columns approximately 0.95 mg of poly(rC) plus poly(rG) was bound per mg of poly(dA-dT). Thus, immobilized heteropolymeric nucleotides (poly(dA-dT)) retain their entire availability for hybridization with complementary sequences.

#### Dimerization

Immobilization induced by UV-light irradiation of nucleic acids probably results in multiple point attachment between the DNA and the cellulose, but the mechanisms are still not completely understood.<sup>13</sup> The nucleic acids form insoluble gels upon UV-light irradiation in solution mostly as a consequence of dimerization between neighboring pyrimidines. The type of dimers formed depends on the base content of the DNA. The formation of thymine dimers (T-T) increases in proportion to the AT base content of the DNA, whereas the formation of cytidine-thymine dimers (C-T) or cytidine dimers (C-C) decreases with increasing AT content. Moreover the total amount of dimers formed is dependent on the base content of the DNA, *i.e.* the higher the AT con-

tent of the DNA the more dimers are formed.<sup>21</sup>

Since we used UV light irradiation to immobilize the DNA, it is likely that dimerization occurred in the DNAs during the preparation of the DNA-cellulose. To test the degree of dimerization induced, the following experiments were performed.

The different nucleic acids (approximately 20  $\mu\text{g/ml}$  in 10 mM NaCl) were irradiated with UV-light as in the preparation of nucleic acid-celluloses, and the changes in the absorbance at 260 nm were determined. A slight drop in the absorbance was regularly observed following UV-light irradiation of the DNAs, the hypochromicity being proportional to the base content of the DNA. At low AT content (*M. lysodeikticus* DNA) the decrease in absorbance was approximately 1 %, whereas the greatest hypochromicity (4 %) occurred with DNA from *Cl. perfringens* with high AT content. These figures indicate that dimerization did take place.<sup>21</sup>

#### The binding of fluorochromes to cellulose-immobilized nucleic acid derivatives

*Polynucleotides.* Table 2 summarizes the amounts of different fluorochromes bound to cellulose-immobilized polynucleotides. Poly-(rA)-cellulose bound similar amounts of acridines (approximately 300  $\mu\text{g/mg}$ ) and the same was true of poly(rG)-cellulose which bound 220 to 240  $\mu\text{g dye/mg}$ . The amount of Hoechst 33258 bound to these two polynucleotide-celluloses was about 30 % lower in each case. Expressed as  $\mu\text{g}$  of dye bound per mg of polynucleotide, the binding capacity of poly(rA) was about 20 % higher than that of poly(rG). However, if the values are expressed as P/D (polynucleotide phosphorus divided by the dye concentration) the amount of acridines bound to poly(rA)-cellulose is only 9 % higher than the amount bound to poly(rG)-cellulose (values not given in the table). We therefore conclude that the difference in the dye binding capacity of poly(rA) and poly(rG) is slight or questionable.

Poly(dA-dT)-cellulose binds 1.5 to 3 times more of the acridines than the homopolymeric nucleotide-celluloses. Furthermore, the different dyes had different affinities for cellulose-

Table 2. The binding of fluorochromes to cellulose-immobilized nucleic acid derivatives.

Nucleic acid cellulose	GC %	( $\mu\text{g}$ fluorochrome)/(mg nucleic acid derivative)			Hoechst 33258
		Acranil	Quinacrine	Quinacrine mustard	
Poly(rA)	—	300	290	290	205
Poly(rG)	—	240	220	230	170
Poly(dA-dT)	—	720	640	460	420
<i>M. lysodeikticus</i>	70	140	130	55	170
<i>E. coli</i>	50	390	290	155	270
<i>Cl. perfringens</i>	31	560	540	380	390
Calf thymus	40	640	480	215	450
Mouse liver	40	660	470	230	420

immobilized poly(dA-dT). The relative amounts of acridines bound were as follows: acranil > Q > QM. The amount of Hoechst 33258 bound to poly(dA-dT)-cellulose was of the same order as that of QM.

**Nucleic acids.** Cellulose-immobilized DNAs with GC contents varying between 31 and 70 % were used to examine the base specificity of the binding between fluorochromes and DNAs. The following conclusions were reached on the basis of the data given in Table 2. The acridines as well as Hoechst 33258 react base specifically with immobilized microbial DNAs. The higher the AT content in the DNA, the greater were the amounts of the fluorochromes bound per mg of DNA. However, the correlation is not rectilinear.

Different proportions of the acridine derivatives were bound to the cellulose-immobilized microbial DNAs, the sequence always being: acranil > Q > QM. The same phenomenon occurred with poly(dA-dT)-cellulose.

The amount of Hoechst 33258 bound to DNAs of different base contents deserves comment. From fluorescence studies in solution it is known that the higher the AT content of the DNA, the stronger is the fluorescence intensity of the solution.<sup>6</sup> At low AT content in DNA-cellulose (*M. lysodeikticus*, 30 % AT) more Hoechst 33258 is bound than any of the acridine derivatives, whereas at high AT content (*Cl. perfringens*, 69 % AT) the reverse is true (Table 2). These findings apparently indicate that the mechanisms by which Hoechst 33258 interacts with nucleic acids are different from those by which the acridines interact.<sup>22,23</sup>

DNA from calf thymus or from mouse liver had similar affinities for the fluorochromes. The greatest amount of acridine bound to mammalian DNAs occurred with the dye acranil and the smallest with QM, whereas the amount of Q bound to mammalian DNAs was intermediate. This is in agreement with the results reported by Modest and Sengupta.<sup>11</sup> They found that the bonds between QM and calf thymus DNA were 25 times as strong as those of Q, but that fewer QM molecules bound to the DNA. The amounts of fluorochromes bound to immobilized mammalian DNAs were generally higher than the amounts bound to immobilized microbial DNAs. The only exception was the very AT rich DNA of *Cl. perfringens*.

#### The binding of proteins to cellulose-immobilized nucleic acid derivatives

The results are seen in Tables 3 and 4. It has been found that more poly-L-lysine was bound to poly(rA)-cellulose than to poly(rG)-cellulose. Furthermore, more poly-L-arginine was bound to poly(rG)-cellulose than to poly(rA)-cellulose. Thus, our results with immobilized polynucleotides correspond with earlier reports concerning the base specific binding of polyamino acids to DNAs.<sup>24</sup> Poly(dA-dT)-cellulose bound as much poly-L-arginine and poly-L-lysine as did poly(rA)-cellulose (Table 3). The different immobilized polynucleotides bound more poly-L-arginine than poly-L-lysine, *i.e.* poly-L-arginine

Table 3. The binding of polyamino acids to cellulose-immobilized nucleic acid derivatives.

Nucleic acid cellulose	GC %	( $\mu\text{g}$ polyamino acid)/(mg poly-L-arginine)	(mg nucleic acid derivative) poly-L-lysine
Poly(rA)	—	260	130
Poly(rG)	—	555	50
Poly(dA-dT)	—	250	150
<i>M. lysodeikticus</i>	70	310	180
<i>E. coli</i>	50	220	230
<i>Cl. perfringens</i>	31	115	450
Calf thymus	40	160	270
Mouse liver	40	160	290

showed higher affinity for polynucleotide-cellulose than did poly-L-lysine.

When polyamino acids reacted with cellulose-immobilized microbial DNAs a clear-cut correlation was observed between the AT content of the DNA and the amount of poly-L-lysine bound, but the relationship was not linear (Table 3). With increasing GC content in the microbial DNAs, the binding of poly-L-arginine also increased; this relationship was linear. DNA from *E. coli* (50 % GC) bound equal amounts of the two polyamino acids.

Differences were also found in the amounts of polyamino acids bound to immobilized mammalian DNAs. The nucleic acids bound

more poly-L-lysine than poly-L-arginine. These findings are in marked contrast to the binding of polyamino acids to polynucleotide-celluloses. Both mammalian DNAs tested, each containing about 40 % GC, bound equal amounts of poly-L-arginine, as was also the case with poly-L-lysine.

The amount of poly-L-arginine (160  $\mu\text{g}$ ) bound to 1 mg of immobilized mammalian DNAs (approximately 40 % GC) is intermediate between the amount bound to DNA from *E. coli* with 50 % GC (220  $\mu\text{g}/\text{mg}$ ) and the amount bound to DNA from *Cl. perfringens* with 31 % GC (115  $\mu\text{g}/\text{mg}$ ). Moreover, the mammalian DNAs bound approximately 280  $\mu\text{g}$  poly-L-

Table 4. The binding of proteins to cellulose-immobilized calf thymus DNA and the effect of proteins on the binding between quinacrine and DNA-cellulose.

Proteins	Protein binding $\mu\text{g}$ protein/mg DNA	Dye binding to DNA-protein complex <sup>a</sup>
Arginine	0	100
Lysine	0	100
Protamine	170	4
Protamine sulfate	0	100
Poly-L-arginine	160	41
Poly-L-lysine	270	7
Poly-L-arginine:Poly-L-lysine		
= 0.33	160	13
= 1.00	180	13
= 3.00	130	14
Histone fractions:		
"Type II A" (intermediate)	270	68
"Type III" (lysine-rich)	310	32
"Type IV" (arginine-rich)	360	68

<sup>a</sup> The inhibition by protein of the capacity of DNA to bind quinacrine is expressed as percent, 100 % being the binding of dye to cellulose-immobilized DNA unexposed to protein.

lysine per mg DNA which is intermediate between the corresponding amounts of poly-L-lysine bound to DNA from *E. coli* (230  $\mu\text{g}/\text{mg}$ ) and *Cl. perfringens* DNA-cellulose (450  $\mu\text{g}/\text{mg}$ ). This would seem to indicate that the base content is of importance in determining the binding capacity of polyamino acids to DNAs. The binding of some other proteins to calf thymus DNA was also tested (Table 4). The very arginine-rich protamine was bound to calf thymus DNA. However, immobilized DNA did not bind arginine or lysine. The amounts of different histone fractions bound to DNA-cellulose varied between 270 and 360  $\mu\text{g}/\text{mg}$  of DNA. The highest amount bound was found with the arginine-rich histone fraction.

#### The effect of proteins on the binding between quinacrine and cellulose-immobilized DNA

It is possible partly or wholly to prevent the binding of fluorochromes to DNA-cellulose by saturating the dye-binding sites in the DNA with proteins (Table 4).

The amino acids arginine, lysine or protamine sulfate did not bind to immobilized DNA from calf thymus and hence did not prevent the binding of Q to the DNA-cellulose. However, protamine bound to immobilized DNA was able almost completely to prevent the binding of Q.

As shown above, calf thymus DNA bound more poly-L-lysine than poly-L-arginine (Table 3). Poly-L-lysine was also much more effective in preventing the binding of Q to the immobilized DNA than was poly-L-arginine. Three different mixtures of poly-L-arginine plus poly-L-lysine were also tested. The amounts of the mixtures bound to DNA-cellulose varied between 130 and 180  $\mu\text{g}/\text{mg}$  of DNA, but the blocking of Q binding was independent of the poly-L-arginine to poly-L-lysine input ratio. The mixtures were, however, more effective in preventing the binding of the dye than was poly-L-arginine alone. The conclusion is that poly-L-lysine is very effective in blocking the dye-binding sites in cellulose-immobilized DNA. This effect is probably due to competition between poly-L-lysine and the dye for binding sites in the AT-rich regions of the DNA.<sup>24</sup>

Polylysine also has a marked effect on the fluorescence intensity of acridine analogs mixed with DNAs in solution. In all cases the polylysine enhanced the fluorescence intensity of the fluorochrome DNA-complex, *i.e.* the effect of DNA on the dye fluorescence was reversed by polylysine.<sup>25</sup>

Results obtained with microbial DNAs (not shown in Table 4) were similar to those with calf thymus DNA, although the blocking was more complete. Poly-L-arginine as well as poly-L-lysine completely prevented the binding of Q to DNA from *M. lysodeikticus*. Poly-L-lysine was also able wholly to block the binding of the dye to DNA from *E. coli*, whereas the blocking with poly-L-arginine was only partial. It is worth noting that hardly any dye adhered to DNA-cellulose columns saturated with polyamino acids, indicating that the polyamino acids *per se* did not bind the dyes under these conditions.

When different histone fractions were bound to the DNA-cellulose the greatest inhibition of the dye-binding capacity was obtained with the lysine-rich fraction "Type III" (Table 4). The inhibition effects obtained with the two other histone fractions were of the same magnitude (68 %).

## DISCUSSION

Although there is now an abundance of data on the effect of nucleic acids on the fluorescence intensity of fluorochromes in solution, less has been written about the quantitative aspects of their binding. Some such studies have been made by ultra-filtration<sup>11</sup> or equilibrium dialysis<sup>23,26-28</sup> and interpreted with the aid of Scatchard plots. O'Brien *et al.*<sup>8</sup> failed to find any DNA base specific fluorescence with quinacrine in solution and this was partly supported by Modest and Sengupta.<sup>12</sup> Similar observations were reported by Müller and Crothers<sup>27</sup> who found that quinacrine shows little base specificity when mixed with DNAs in solution. On the other hand, our previous findings on the base specific effect of DNAs on fluorescence intensity of quinacrine mustard in solution<sup>2,5,29</sup> and similar discoveries by other workers<sup>1-3</sup> indicate base specific interactions between fluorochromes and nucleic acids.

Hence the experimentation needed to be extended.

The use of immobilized nucleic acids seems to give a more direct method, which allows a quantitation of the fluorochromes bound to the DNAs. The experimental procedure involves UV-light irradiation which brings about pyrimidine dimerization of the DNA.<sup>18,21</sup> The UV-light produces chemical and physical changes in the DNA. The absorption maximum of the DNA is reduced and the pyrimidine dimers produce conformational changes in the DNA. These changes have to be taken into consideration when the results in this paper are compared with results obtained by other methods.

Our data reveal that the quantity of the fluorochromes acranil, quinacrine, quinacrine mustard, and Hoechst 33258 bound to DNA is strongly dependent on the base content of the nucleic acid. This finding is different from those presented by others.<sup>8,11,23,27,28</sup> Furthermore, in a recent paper Latt *et al.*<sup>26</sup> reporting Scatchard plots and circular dichroism data, concluded that the DNA base composition has very little effect on quinacrine binding affinity. It is likely that the different results pertaining to the binding affinity presented by others and by us may be due to the differences in methodology.

The AT base specific binding of fluorochromes to cellulose-immobilized DNA shown in this paper may partly be a result of the dimerization of the DNA. The thymine regions in the DNA may bind more or fewer dye molecules than the other regions, due to denaturation of the DNA in the dimerized regions. Earlier studies with fluorochromes and DNAs in solution show that the fluorescence intensity of the fluorochromes is affected by the strandedness of DNA.<sup>5,29,30</sup> The decrease in the absorption maximum of the DNA after irradiation indicates that DNA undergoes conformational changes when exposed to UV-light (*cf.* Results). Although the UV-light irradiation caused damage to the DNA, the cellulose matrix was probably able to partly prevent the conformational changes since even AT-rich cellulose-immobilized DNAs were able to bind fluorochromes. The results in this paper show base specific binding between the fluorochromes and DNAs. This is supported by studies recently reported

by Müller *et al.*<sup>23,27,28</sup> They studied the base specific reactions between DNAs and intercalating as well as non-intercalating DNA ligands. Strong AT base specific binding between the dye Hoechst 33258 and DNA was found and the compound seems to need three AT pairs for the binding site.<sup>23</sup>

Our results with proteins bound to cellulose-immobilized DNAs confirm previous studies indicating base specificity of the binding of polyamino acids and histone fractions to DNA.<sup>24</sup> Poly-L-arginine preferentially bound to GC-rich regions, whereas poly-L-lysine bound to AT-rich regions in the DNA. Furthermore the proteins blocked the binding between fluorochromes and DNA, thereby reducing the binding sites of the dyes to DNA. This competition is not surprising since it is known that proteins are bound to the phosphate groups of the DNA backbone.<sup>31</sup> The proteins also occupy either the minor and/or the major grooves of the DNA.<sup>31</sup> Thereby they not only inhibit the ionic bindings between the fluorochromes and the DNA but are also able to compete for the intercalation between dyes and the DNA. The polyamino acids show base specificity when bound to DNA. It is therefore natural that poly-L-lysine and lysine-rich histone fractions have the greatest effect on fluorochromes since they bind preferentially to AT-rich sequences,<sup>32</sup> whose affinity for these fluorochromes is strongest. Our results are consistent with those of Weisblum *et al.*<sup>25</sup> and Latt and Gerald<sup>33</sup> as regards the ability of polylysine to compete for dye-binding to DNA.

It was previously thought that differences in the base content of the DNA could explain the Q-banding of metaphase chromosomes.<sup>34</sup> However, our quantitative data suggest that proteins may have a substantial influence on the *in situ* fluorescence pattern. Similar conclusions could be drawn from studies of the density of nucleoprotein along the metaphase chromosomes.<sup>35</sup>

It may be too early to draw conclusions about the banding of metaphase chromosomes on the basis of binding studies in columns or in solution. Only further work will disclose whether different amounts of fluorochromes bind to different parts of the chromosomes and thereby contribute to banding. However, the present study may open the door to better

understanding of the pattern along metaphase chromosomes since there appears to be a clear-cut interrelation not only between DNA base composition and the intensity of fluorescence, but also between DNA base composition, affinity for different proteins, binding capacity for fluorochromes and finally the intensity of fluorescence.

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