INTERCALATING AGENTS AS PROBES OF THE SPATIAL RELATIONSHIP BETWEEN CHROMATIN COMPONENTS

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Summary: Specific histone lysine residues are methylated by a chromatin-bound methyltransferase in cell nuclei incubated with S-adenosylmethionine, as monitored by uptake of radiomethyl. The stimulation of this process by aromatic cations via intercalation into the DNA double helix suggests a valuable new approach to investigating DNA-histone-nonhistone protein interactions in intact chromatin.

In spite of an explosive increase of information on the various components of eukaryotic chromatin (1-4), only very little insight into the structural arrangement of component nucleic acid and protein moieties has been gained. The enormous complexity of its structure is suggested by reports indicating tertiary structure in DNA (5), and the presence of a vast number and variety of non-histone proteins whose relative quantities vary with different conditions in the cell (2,4).

Recent information on the arrangement of histones on DNA indicates some type of semiconservative pattern of histone distribution, with newly synthesized histones attached to the new DNA strand, preexisting ones to the old strand (6), and oligomeric complexes of histones f2a1 and f3, and histones f2a2 and f2b in regularly repeating clusters (7). Models of histone-DNA binding should take into consideration that all histones have well-defined polar and nonpolar segments. An attractive model proposed by Zicardi and Schumaker (8) assumes that the polar segments are involved primarily with DNA binding whereas the nonpolar regions are responsible for protein-protein interactions with nonpolar segments of other histones. Inaccessibility of histone lysine residues to chemical reagents (9) suggests that for all

histones the entire molecule is interfaced to either DNA or other proteins.

Notwithstanding this inavailability, the histone lysine methyltransferase is able to insert methyl groups into the ε-amino groups of specific internal lysine residues located on the polar, basic segments of histones f2a1 and f3. Since the histone lysine methyltransferase is firmly integrated into chromatin, and can be extracted only with sodium desoxycholate, it belongs to the class of nonhistone proteins. In view of the tight binding which presumably exists between the polar histone regions and DNA, we inferred that the activity of the endogenous lysine methyltransferase in isolated nuclei was probably sensitive to agents known to affect DNA conformation.

Materials and Methods. Nuclei from 0.5 g wet weight fresh rat liver were incubated in the presence of S-adenosylmethionine-methyl-^{3}H (New England Nuclear, 8Ci/mmole) as previously described (10), as were subsequent isolation of histones and measurement of radioactivity. The specific activity of controls containing no added agents was 90,000 cpm/mg histone. Protein was determined as described by Hartree (11). DNA concentration was found to be 0.88 ± 0.04 mg/g liver by extraction of chromatin from the same source with hot 0.5 M perchloric acid and measurement of the absorption of the extract at 260 nm. Calf thymus DNA (Sigma Type V) was used as standard after similar extraction. The dinitroaniline reporter, N-(2(2,4-dinitroanilino)-ethyl)- N,N,N',N',N' - pentamethylpropane - 1,3-diammonium dibromide, was a gift of Dr. E. J. Gabbay. 4.7-dimethyl NMP (4,7-dimethyl-N-methyl-1,10-phenanthrolinium chloride) and 4,7-diphenyl NMP were synthesized as previously described (12). Miracil D was a gift of Dr. M. A. Lea, New Jersey Medical School; Ethidium Bromide was a gift of Dr. M. Cory, Stanford Res. Institute.

Results and Discussion. We have reported earlier that a number of carcinogens inhibit histone lysine methylation in nuclei (10), and now report that, in contrast, this process is stimulated in the presence of agents which intercalate between the base pairs of the DNA helix, either as the free polynucleotide in solution (12-15) or in chromatin, with no concommitent dissociation of pro-

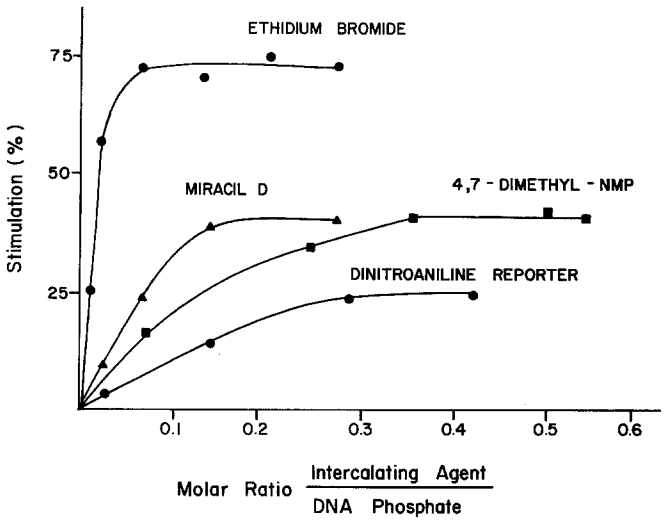

Figure 1. Effect of intercalating agents on incorporation of radiomethyl into histones of rat liver nuclei incubated in the presence of S-adenosyl-methionine-^{3}H. Each value stated is the average of at least two experiments, and duplicates were run in each experiment. Experimental variation of these values was ± 4% from the mean.

tein (15-18). The distribution of radiomethyl among various histone fractions is apparently the same in the presence or absence of the intercalating agent (19). The stimulation observed increases linearly with increasing concentration of intercalating agent initially, eventually reaching a constant maximum value characteristic of the agent present (Fig. 1 and Table 1). Analogous behavior is observed for the viscosity and induced circular dichroism of DNA-intercalating agent complexes (12). An estimate of the number of primary binding sites per nucleotide (n) determined from data such as that shown in Fig. 1 agrees well with values of n determined by spectrophotometry (15,17,18).

The majority of intercalating agents have both aromatic and cationic functions in their molecular structure. However, if the structure includes bulky substituents, as in the case of 4,7-diphenyl NMP, intercalation is prevented on steric grounds (12,20). This agent is observed to cause marked

TABLE I

STIMULATION OF HISTONE METHYLATION BY INTERCALATING AGENTS

Agent	Maximal Stimulation (% of Control)	n*
Acridine Orange	22	0.011
Actinomycin D	62	0.025 (0.027)
4,7-Dimethyl NMP	40	0.20
4,7-Diphenyl NMP	0**	--
Dinitroaniline Reporter	22	0.170 (0.250)
Ethidium Bromide	72	0.036 (0.033)
Miracil D	40	0.125
Proflavine	71	0.061
Spermine	0	--
Spermidine	0	--
N,N-hexamethylpropane-1,3-diammonium Dibromide	0	--
Tri-α-L-lysine	0	--

* Maximum number of primary binding sites per nucleotide of nuclear DNA. Values in parentheses were reported by others from spectrophotometric determinations made on chromatin under conditions of ionic strength similar to those above for actinomycin D (15), the dinitroaniline reporter (12) and ethidium bromide (16).

** 45% inhibition of histone methylation was observed in the presence of this agent.

Experimental variation of n values from stimulation experiments was ± 10% from the mean.

inhibition of histone lysine methylation in nuclei (Table I), a demonstration that the stimulation observed with intercalating agents is not solely due to electrostatic interaction between the cations and the DNA-phosphate anions. Furthermore, non-aromatic cations have no effect on the chromatin-bound methyltransferase at any concentration (19). The observation that stimulation occurs

in the presence of Actinomycin D, an intercalating agent with no charge at or above pH 7 (21) again demonstrates that simple electrostatic interactions are insufficient to produce this effect. The possibility that stimulation arises from interaction between intercalating agents and the methyltransferase enzyme itself is negated by our observation that these agents show no stimulatory effect on the methylation of free histone by the enzyme solubilized from nuclei, at any intercalating agent: enzyme concentration ratio (19).

Ethidium bromide intercalation into various types of chromatin (22) has been found to be closely correlated with transcriptional activity, suggesting that its binding is limited to active parts of the chromatin. The strong stimulation of histone methylation by this dye would therefore suggest that the methylation process can be stimulated in euchromatin. It is intriguing to consider the possibility that stimulation is due to the turning-on of formerly nonexistent methylation in euchromatin.

Our results show that stimulation of histone methylation in nuclei offers a further diagnostic test for determining whether a particular agent intercalates into the DNA helix. The data presented suggest, furthermore, that DNA, polar histone segments and histone lysine methyltransferase (a nonhistone chromosomal protein) are situated in close proximity within the molecular framework of chromatin, since small changes in DNA conformation have a pronounced effect on the histone methylation process.

It has previously been proposed that a DNA-protein recognition process may exist via the intercalation of aromatic aminoacid residues into DNA (23, 24), although the ability of aromatic aminoacid derivatives to intercalate has recently been questioned (25). Our observation that decarboxylated aromatic aminoacids also stimulate histone lysine methylation in nuclei (19) supports other evidence that such molecules, and possibly aromatic aminoacid residues in proteins, are able to intercalate into the DNA helix.

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