Chromosoma (Berl.) 35, 84–98 (1971)

Constitutive Heterochromatin in *Microtus agrestis*: Binding of Actinomycin-D and Transcriptional Inactivity

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Received June 3, 1971 / Accepted July 5, 1971

Abstract. The constitutive heterochromatin of the field vole, *Microtus agrestis*, fails to incorporate ^{3}H-uridine. It must be considered to be transcriptionally inactive. Nevertheless, in both living and fixed cells constitutive heterochromatin binds labelled actinomycin-D (^{3}H-AMD) at the same rate or at even a higher rate than euchromatin per unit DNA. Extraction of histones by acids leads to an increase of the AMD-binding capacity of the entire chromatin. The number of free AMD-binding sites is found to be largely independent of the degree of chromatin condensation and heteropycnosis.—It is suggested that AMD-binding to constitutive heterochromatin reflects a genetic inactivity which is not necessarily connected with a repressive function of histones and with chromatin condensation. In this respect a distinct difference seems to exist between constitutive and facultative heterochromatin.

Introduction

It is generally accepted that heterochromatization is a means by which larger segments of the genome become genetically repressed in higher organisms. Heterochromatin was originally characterized by heteropycnosis during interphase (Heitz, 1929) and genetic inertness (Muller and Painter, 1932; Heitz, 1933). Late replication of DNA was found to be a further feature (Lima-de-Faria, 1959; Taylor, 1960). A distinction should be made between two types of heterochromatin, "constitutive" and "facultative" (see Brown, 1966).

Facultative heterochromatin can be regarded as being chromatin in a temporarily repressed state. It may arise, for instance, during development, well known examples being the heterochromatization of a haploid chromosome set in male mealy bugs (Schrader, 1929; Hughes-Schrader, 1935; Brown and Nur, 1964) and that of one X-chromosome in female mammals (Lyon, 1966, 1968). Obviously, facultative heterochromatization is connected with a mechanism of inactivation which is not accompanied by any change of genetic composition.

Less is known about the nature and function of constitutive heterochromatin. Evidence for its transcriptional inactivity in mammals has been presented (Hsu, 1962; Sieger *et al.*, 1970). Recent studies indicate an enrichment of highly repetitive DNA as a significant feature of

constitutive heterochromatin (Yunis and Yasmineh, 1970; Arrighi *et al.*, 1970).

In the present study we investigated the actinomycin-binding property of mammalian constitutive heterochromatin. Actinomycin-D is known to bind with double-stranded DNA, thereby inhibiting DNA-dependent RNA synthesis (Reich and Goldberg, 1964). It has been stated that the degree of actinomycin-binding is influenced by the state of repression of chromatin in various cell types (Brachet and Hulin, 1969; Ringertz and Bolund, 1969; Ringertz *et al.*, 1969; Berlowitz *et al.*, 1969).

For the purposes of this study we used the European field vole, *Microtus agrestis*, for the following reasons: 1. This species offers the unique advantage that most of the constitutive heterochromatin is accumulated on the two very long sex chromosomes (Wolf *et al.*, 1965; Schmid, 1967) which in certain tissues form large chromocenters (Matthey, 1950; Schmid 1967; Pera, 1969). 2. In a previous paper we demonstrated that the constitutive heterochromatin of *Microtus agrestis* fails to synthesize RNA (Sieger *et al.*, 1970).

The results of our present study suggest that constitutive heterochromatin, in contrast to facultative heterochromatin contains a primary, transcriptionally inactive DNA and does not represent a repressed state of chromatin. Some of the results of the present report have been published preliminarily (Sieger and Garweg, 1971).

Materials and Methods

The field voles, *Microtus agrestis*, were obtained from our breeding colony. The investigations were carried out on cells from the cerebral cortex because of their well formed and large chromocenters. In 70% of the nuclei two separated chromocenters are discernible. These are fused together in 30% of the nuclei and appear as one double-sized heterochromatin body (Pera, 1969). Only males were used because in males the chromocenters are built up solely of constitutive heterochromatin. Thus any influence by facultative heterochromatin, as present in females, was ruled out. Binding of tritiated actinomycin-D (AMD) was tested in living cells as well as in air-dried and in fixed cells without and with extraction of histones. The incorporation of tritiated uridine was taken as a control for intact transcriptional activity of the cells.

Living Cells. Parts of the cerebral cortex were fragmented and suspended in Hanks' solution containing actinomycin-D-^{3}H (specific activity 8.4 Ci/mM; Schwarz Bio Research) at a concentration of 10 μCi/ml. Incubation was carried out for 30 min, 1 and 2 hrs at 37° C. Following incubation the cells were washed with unlabelled Hanks' solution to dilute the radioactivity. They were then centrifuged, resuspended in fresh Hanks' solution and again centrifuged. The washing procedure was repeated twice in order to remove all unbound ^{3}H-AMD. Finally the cells were fixed with 10% formalin/96% ethanol 1:1 for 30 min and then transferred to gelatine-coated slides (0.5% gelatine, 0.05% chrome alum) and air-dried. After washing in distilled water the slides were autoradiographed. Several cell suspensions were labelled with uridine-5-^{3}H (specific activity 19.4 Ci/mM; concentration 50 μCi/

ml; RCC Amersham). These suspensions were treated in the same manner, but incubation time was restricted to 30 min and fixation was carried out with ethanol-acetic acid 3:1.

Fixed Cells. Smear preparations from the cerebral cortex were made on gelatinized slides. These were immediately dipped into one of the following fixatives: a) 10% neutral buffered formalin, b) 3% glutaraldehyde, c) methanol abs., d) 96% ethanol. Several preparations were immediately air-dried without fixation. After 30 min the fixed cells were washed in distilled water and dried in air. Three drops of actinomycin-D-^{3}H (concentration 10 μCi/ml) were placed over the cells on the slide. The slides were kept in a moist chamber for 30 min at room temperature. Incubation was followed by washing in distilled water and air drying.

Histone Extraction. Smear preparations were fixed and extracted with 45% acetic acid (pH 1.4) for 30 min at room temperature. Those to be used for further extraction were transferred to 0.5 N sulphuric acid for 30 min at 0° C. After rinsing in distilled water and air drying the slides were incubated in ^{3}H-AMD in the same manner as described for fixed cells. It has been shown by Dick and Johns (1968) that 45% acetic acid extracts approximately 55% of the total histone from calf thymus in deoxyribonucleoprotein preparations. Mainly fractions f1 (lysine rich) and f2a2 (intermediate) are removed. Treatment with sulphuric acid leads to nearly complete extraction of all histones (Murray, 1966).

Autoradiography. The slides were coated with either Kodak AR 10 stripping film or Kodak NTB-2 liquid emulsion, diluted 1:1. Exposure periods varied from 48 hrs to 21 days. The slides were developed in Kodak D-19 for 4 min. Developing, fixing and washing procedures were carried out at 4° C to prevent the film layer from producing bubbles which often occurred in the presence of ^{3}H-AMD.

Evaluation. After autoradiography the slides were stained with pararosaniline-methyl green according to the method described previously (Pera and Wolf, 1967; Sieger *et al.*, 1970) or left unstained. After a photographic record of the cells had been made, the stripping film was removed and silver grains bleached out (7.5% potassium ferricyanide 25 min, 20% sodium thiosulphate 10 min). The cells were then stained by the Feulgen technique and rephotographed. From autoradiographs coated with NTB-2 emulsion and stained with pararosaniline-methyl green, grain counts were taken. Only nuclei of neurocytes covered by not more than 80 silver grains and having two clearly separated chromocenters were evaluated. In order to compare the grain counts of eu- and heterochromatin, these were corrected for DNA content. The proportion of DNA within the heterochromatin of the sex chromosomes was taken to be 17% of the total genome (Schmid, 1967).

Results

I. Incorporation of ^{3}H-Uridine

Brain cells are still capable of synthesizing RNA after incubation in Hanks' solution at 37° C for two hours. The cells incorporate ^{3}H-uridine at the same rate when it is offered during the last half hour as when they are directly incubated in ^{3}H-uridine for only half an hour (Fig. 1). This serves as a control, ensuring that the cells are still alive after being kept for 2 hours in suspension. As can be seen from Fig. 1, eu- and heterochromatin differ markedly in their incorporation of uridine. In all types of brain cells the silver grains are localized almost exclusively over the euchromatin, whereas the chromocenters formed by constitutive

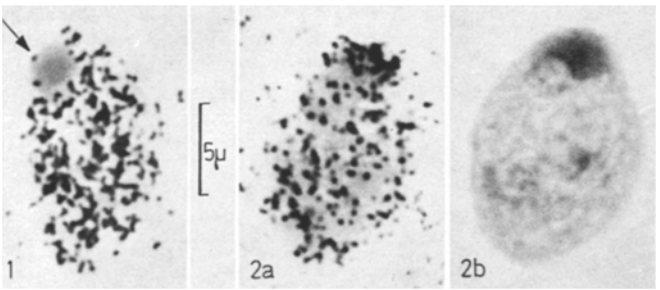


Fig. 1. ^{3}H-uridine labelling: Autoradiography of a cell nucleus from brain cell suspension (50 μCi/ml; incubation for 30 min, exposure for 21 days). Pararosaniline and methyl-green stain. The fused chromocenters (arrow) are not labelled

Fig. 2a and b. ^{3}H-AMD labelling of living cells. a Autoradiography of a cell nucleus from brain cell suspension (10 μCi/ml; incubation for 2 hours; exposure for 21 days). b Same nucleus after grain removal and Feulgen stain. Chromocenters (fused) are labelled

heterochromatin are practically free of grains. This transcriptional inactivity of heterochromatin has been described in detail in a former paper (Sieger *et al.*, 1970). The grain counts of eu- and heterochromatin show a ratio of 49:1, which corresponds to a ratio of 10:1 when related to DNA quantity. This ratio may well be even higher since some of the silver grains over the heterochromatin presumably originate from overlapping euchromatin or nucleolar material.

II. Binding of ^{3}H-AMD to Living Cells

After incubation of suspended brain cells in ^{3}H-AMD for 30 min, 1 and 2 hours, the cell nuclei are distinctly labelled whereas the cytoplasm remains almost free of grains. Contrary to the labelling pattern after ^{3}H-uridine, the heterochromatin is heavily labelled (Fig. 2). The euchromatin regions are also labelled but somewhat more weakly. The nucleoli incorporate no or only small amounts of ^{3}H-AMD. Grain counts indicate that the binding of ^{3}H-AMD is directly correlated to the quantity of DNA of the different nuclear regions. The ratio of AMD-binding of euchromatin to that of heterochromatin is practically 1:1 when corrected for the amount of DNA (Table 1). The degree of condensation itself is without influence on the amount of incorporated ^{3}H-AMD. Even such chromocenters which appear as very strongly condensed heterochromatin in Feulgen preparations show a corresponding density of grains. It should be pointed out that no qualitatively detectable difference in AMD-binding exists between the 30 min, 1 hour and 2 hour values in respect of the ratio of euchromatin to heterochromatin. The quantitative data

Table 1. *Incorporation of tritiated uridine (50 μCi/ml) and actinomycin-D (10 μCi/ml) in neurocytes of the cerebral cortex of male field voles (Microtus agrestis). Eu euchromatin; Het heterochromatin. Ratio Eu/Het ± standard deviation of the mean ($s/\sqrt{n}$). Exposition times in all cases = 7 d, except for acetic acid = 2d.*

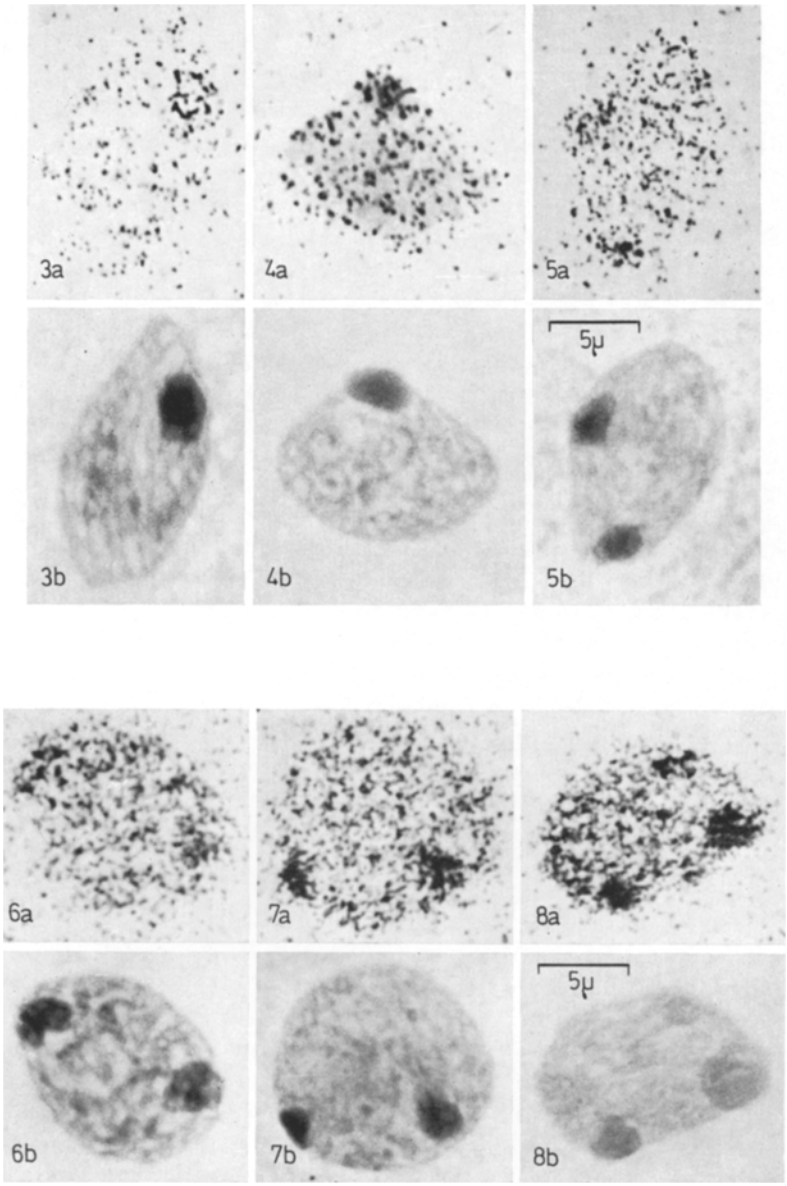
ıbel	Treatment	Number of cells	Grains/Eu absol.	Grains/Eu %	Grains/Het absol.	Grains/Het %	Ratio Eu/Het	Ratic Eu/H per ι DNA
[-uridine (30 min)	Live suspension	25	78.4	98	1.6	2	49.0±2.46	9.80
[-AMD (30 min)	Live suspension	19	34.3	82	7.6	18	4.5±0.73	0.90
[-AMD (2h)	Live suspension	27	52.3	84	10.0	16	5.2±0.67	1.04
[-AMD (30 min) after fixation	Formalin 10 %	38	35.3	86	5.8	14	6.1±0.68	1.22
	Glutaraldehyde 3 %	42	50.8	78	14.4	22	3.5±0.42	0.70
	Methanol absol.	15	49.7	80	12.4	20	4.0±0.51	0.80
	Ethanol 96%	12	46.7	81	10.9	19	4.3±0.77	0.86
	Air-dried	20	52.6	76	16.6	24	3.2±0.35	0.64
[-AMD (30 min) after extraction of histones	Acetic acid 45 %	40	65.0	83	13.3	17	4.9±0.81	0.98

for 30 min and 2 hours (see Table 1) confirm this. Both fractions of chromatin bind AMD at a similar rate.

III. Binding of ^{3}H-AMD to Air-Dried and Fixed Cells

Brain cells which have been air-dried or fixed before incubation reveal principally the same ^{3}H-AMD labelling pattern as living cells (Figs. 3–8). The chromocenters are, due to their higher DNA value, much more strongly labelled than the euchromatin. There are, however, some quantitative differences, depending on the kind of fixation. Table 1 summarizes the results of the grain counts.

Among all the applied treatments, only fixation in 10% formalin tends towards a slightly stronger ^{3}H-AMD-binding of the euchromatin. Corrected for DNA quantity the relation between euchromatin and heterochromatin is about 1:1.2 (Table 1). Formalin-fixed nuclei are in general somewhat more weakly labelled than those that have been fixed by other methods.



Figs. 3–8a and b. ^{3}H-AMD labelling of fixed cells. Cell nuclei from brain smear preparations: a Autoradiography (10 μCi/ml; incubation for 30 min). Exposure for 7 days (3–5) and 21 days (6–8). b After grain removal and Feulgen stain. Figs. 3 and 6. Neutral buffered formalin (10%). Fig. 4. Ethanol (96%). Fig. 5. Methanol abs. Fig. 7. Glutaraldehyde (3%). Fig. 8. Air drying

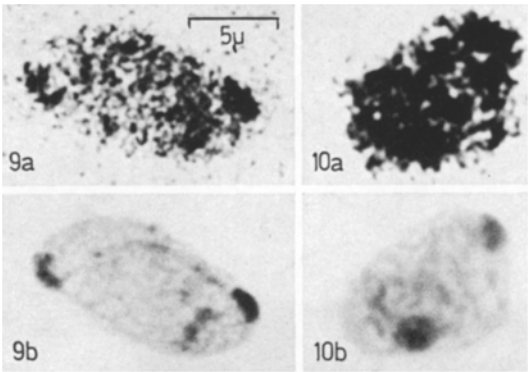
In nuclei fixed with ethanol or methanol the ratio of the ^{3}H-AMD-binding capacity of euchromatin to heterochromatin is slightly in favour of heterochromatin (Table 1, Figs. 4 and 5). This tendency is enhanced in air-dried and glutaraldehyde fixed cells (Table 1, Figs. 7 and 8; compare with Fig. 6, which shows a formalin-fixed nucleus at the same exposure time).

These results show that the mode of pretreatment and fixation influences the ^{3}H-AMD-binding ratio of euchromatin to heterochromatin to some extent. The question arises, therefore, as to what sort of treatment best reflects the conditions *in vivo*. In the case of the living cells it should be considered that the uptake of AMD may depend upon numerous factors, especially on penetration conditions (see Bolund, 1970). One could argue that air-dried cells reflect the conditions *in vivo* best. In air-dried preparations the AMD-binding of heterochromatin is somewhat higher than that of euchromatin. Of the various fixatives applied, glutaraldehyde gives results nearest to those obtained by air-drying. Furthermore, glutaraldehyde preserves the DNP complex to a high degree in its native condition (Sabatini *et al.*, 1963; Habeeb and Hiramoto, 1968; Richards and Knowles, 1968). Formalin-fixed cells generally show a weaker AMD-binding. This may be due, to some extent, to a denaturation of proteins, but may be chiefly caused by the negative chemographic effect of formalin on the photographic emulsion (Rogers, 1967). It should be emphasized, however, that different treatment of the cells causes only minor variations in the AMD-uptake ratio of euchromatin to heterochromatin. This ratio is always near 1:1. Hence, the correlation of AMD-binding to quantity of DNA is the main reason for the observed differences in labelling of euchromatin and heterochromatin.

IV. Binding of ^{3}H-AMD after Extraction of Histones

Extraction of lysine-rich histones by 45% acetic acid leads to a drastic enhancement of ^{3}H-AMD-binding of the entire chromatin (Figs. 9 and 11; compare Figs. 3–5). The number of silver grains is about 6 times higher than after formalin, and at least 4 times higher than after glutaraldehyde fixation. The grain counts show further that euchromatin and heterochromatin bind roughly equal amounts of ^{3}H-AMD per unit DNA (Table 1).

Compared to the values after air drying or glutaraldehyde fixation, the euchromatin seems to gain a little more than the heterochromatin in AMD-binding capacity after the acetic acid treatment. This treatment, however, makes the chromatin in general coarser, and the chromocenters appear very compact (compare Fig. 9b with Fig. 6b). Silver grain counts are therefore less reliable. Furthermore, the β-absorption of the very



Figs. 9 and 10a and b. ^{3}H-AMD labelling of brain cells after extraction of histones. a Autoradiography (10 µCi/ml; incubation for 30 min, exposure for 7 days). b After grain removal and Feulgen stain. Fig. 9. Fixation and extraction with 45% acetic acid. Fig. 10. Further extraction with 0.5 N sulphuric acid

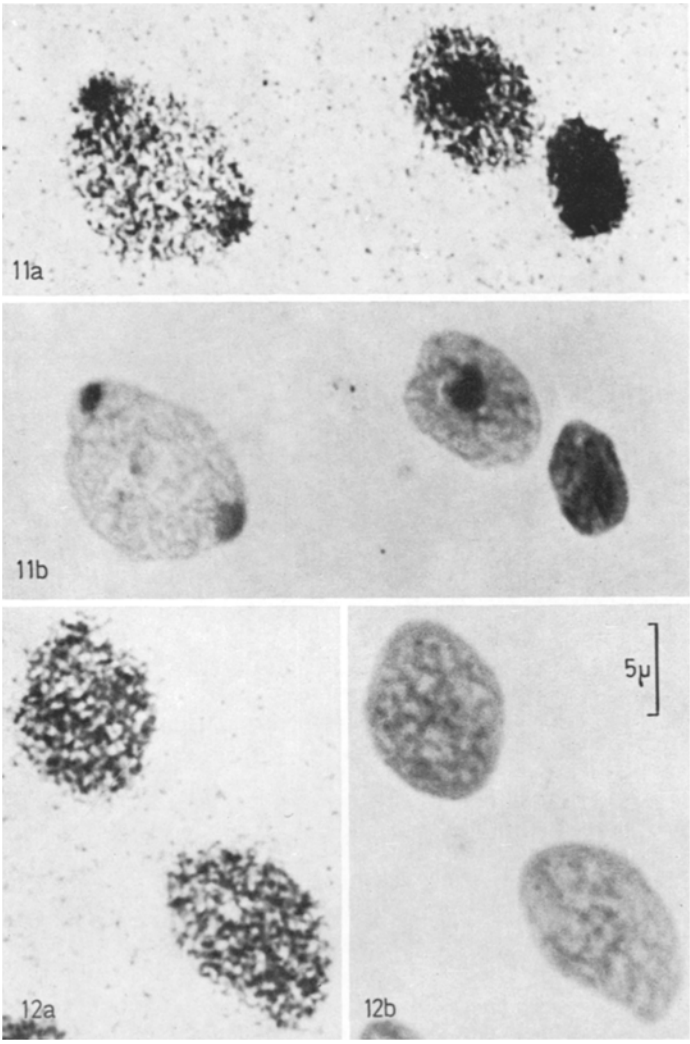
strongly condensed chromocenters may lead to a decrease in the number of grains.

The almost complete extraction of all histones by additional treatment with 0.5 N sulphuric acid leads to a further increase of ^{3}H-AMD-uptake (Fig. 10). Euchromatin and heterochromatin seem to be similarly involved. Quantitative data could not be recorded in this case.

V. Chromatin Condensation and Binding of ^{3}H-AMD

The above results demonstrate that in the case of heterochromatin, its high degree of condensation is no obstacle for binding ^{3}H-AMD. Moreover, different degrees of condensation of the euchromatin have no noticeable effect on the rate of AMD-uptake. In all preparations the small, highly condensed nuclei of glial cells are much more intensively labelled than the large and less condensed nuclei of nerve cells (Fig. 11). The absolute number of grains is, however, about the same in both cell types, which corresponds to their equal DNA content. This again demonstrates that the AMD-binding is chiefly dependent on the quantity of DNA of a given nuclear area and is not related to chromatin condensation itself.

Some cell nuclei in our preparations do not reveal any heteropycnotic structures, hence the constitutive heterochromatin is in an isopycnotic state. In such cell nuclei an even distribution of incorporated ^{3}H-AMD is observed (Fig. 12).



Figs. 11–12a and b. Chromatin condensation and AMD-binding: Brain cell nuclei after labelling with ^{3}H-AMD. Fixation as in Fig. 9. a Autoradiography (10 μCi/ml; incubation for 30 min; exposure for 7 days). b After grain removal and Feulgen stain. Fig. 11. Cell nuclei with different degrees of chromatin condensation. Fig. 12. Isopycnotic state of the heterochromatin

Discussion

Our results in respect of ^{3}H-uridine incorporation show, as do the results reported in our previous paper (Sieger *et al.*, 1970), that the

constitutive heterochromatin of *Microtus agrestis* is transcriptionally inactive. In the present investigation we also demonstrated that contsitutive heterochromatin is capable of binding ^{3}H-AMD to an equal or a perhaps even greater extent than euchromatin. After htisone extraction, additional AMD-binding sites become available in the entire chromatin. The binding of AMD per unit DNA is largely independent of the degree of condensation of the chromatin.

AMD-binding and Transcriptional Inactivity

AMD is specifically bound to DNA not only in isolated DNP preparations but also to DNA *in vivo* (Brachet and Ficq, 1965; Ebstein, 1967; Camargo and Plaut, 1967). It has been suggested that the capacity of binding AMD is correlated to genetic activity because AMD can only occupy those sites of the DNA molecule which are not repressed and hence are being transcribed (Brachet and Hulin, 1969; Ringertz and Bolund, 1969; Berlowitz *et al.*, 1969). The findings on the relation of AMD-binding to genetic activity published up till now are, however, non-uniform.

Brachet and Hulin (1969, 1970) found that the capacity of AMD-binding decreases with differentiation in certain cells (e.g. spermatocytes). Ringertz *et al.* (1969) observed a good correspondence between RNA synthesis and AMD-binding in human lymphocytes *in vitro*. On the other hand, structures of high RNA synthesis, such as the loops of lampbrush chromosomes and puffed regions of polytene chromosomes do not show an increased AMD-binding (Ebstein, 1967; Camargo and Plaut, 1967; Desai and Tencer, 1968). In these instances AMD-uptake is presumably in direct proportion to DNA quantity. It must be considered, however, that these special structures are highly decondensed and that quantitative evaluations are hence not possible.

Berlowitz *et al.* (1969) investigated the facultative heterochromatin of male mealy bugs. They demonstrated that the heavily condensed paternal chromosome set in somatic cells of this species, i.e. the facultative heterochromatin, binds far less AMD than the euchromatin. Simard (1967) found, on the contrary, an increased AMD-binding of condensed chromatin particles in cell nuclei of hamster fibroblast cultures using electron microscopic autoradiography. It seems conceivable that the dense nuclear regions of hamster fibroblast represent constitutive heterochromatin (Schmid, 1967). The increased AMD-binding may be due to a higher DNA concentration. These findings of Simard accord well with ours in respect to the constitutive heterochromatin of *Microtus agrestis*.

Some of the differences quoted may be due to different treatments of cells. Nevertheless, a correlation of AMD-binding to genetic activity

actually does exist in certain instances of differentiating or activated cells as well as in cells with facultative heterochromatin. In these cases it seems that AMD cannot be bound to DNA in a repressed state.

The constitutive heterochromatin presents a special case. Its AMD-binding capacity is directly correlated to the amount of DNA, and its transcriptional inactivity does not prevent the binding of AMD. This suggests that the transcriptional inactivity is not caused by a repression mechanism which at the same time is a hindrance to AMD-binding, as seems to be the case with facultative heterochromatin or with inactivated euchromatin. Therefore, the constitutive heterochromatin may be regarded as being primary, genetically inactive and its DNP complex may be basically different from that of facultative heterochromatin.

One peculiarity of constitutive heterochromatin is its high content of repetitive nucleotide sequences. This has been shown for a number of different mammals (Yunis and Yasmineh, 1970; Pardue and Gall, 1970; Jones, 1970; Jones and Robertson, 1970; Yasmineh and Yunis, 1970, 1971a). Since in many cases the base composition of this type of DNA differs from the main DNA, it can be separated by CsCl gradient centrifugation as so-called satellite DNA. In *Microtus agrestis* only a very low percentage of total DNA is present as satellite DNA (Hennig and Walker, 1970). Nevertheless the constitutive heterochromatin of *Microtus agrestis* is rich in repetitive DNA as shown by *in situ* annealing experiments (Arrighi *et al.*, 1970). The repetitive DNA amounts to about 26% of total DNA (Yasmineh and Yunis, 1971b). This high content of repetitive DNA of constitutive heterochromatin may be connected with its transcriptional inactivity and with its specific behaviour towards AMD.

Other points also deserve mention. Since AMD is bound to guanine one could argue that constitutive heterochromatin is relatively rich in guanine-cytosine pairs. Hennig and Walker (1970) and Yasmineh and Yunis (1971b), however, were not able to demonstrate a corresponding GC-rich fraction of DNA in *Microtus agrestis*. Our results can be interpreted rather in this way, that euchromatin and constitutive heterochromatin bind AMD to the same extent and that constitutive heterochromatin in *Microtus agrestis* is of average base composition.

Simard (1967) discussed the possibility that an increased AMD-binding to condensed chromatin is based on the complete double-strandedness of inactive chromatin, whereas euchromatin is partially single-stranded. This interpretation is not very probable. Aside from the question of strand separation with transcription (*e.g.*, Florentiev and Ivanov, 1970; Riley, 1970), this factor can be neglected, since presumably only a small part of the genome of a cell is active.

With regard to the kinetics of AMD-binding, it should be pointed out that in our material, eu- and heterochromatin do not differ in their rate of AMD-binding after short (30 min) and prolonged (2 hours) incubation. Ringertz *et al.* (1969) found a difference in this respect between activated and inactivated lymphocytes.

AMD-Binding, Histones and Chromatin Condensation

Desai and Tencer (1968) demonstrated a 2–3 fold increase of the AMD-binding capacity of polytene chromosomes of *Chironomus* salivary glands after trypsin treatment. They concluded that trypsin attacks chromosomal proteins and in this way makes additional binding sites available for AMD. The opposite effect occurs after histone application. Berlowitz *et al.* (1969) found in mealy bug cells a distinct increase of AMD-binding after acid extraction of histones in the entire chromatin, the facultative heterochromatin showing a higher increase relative to the euchromatin.

In *Microtus agrestis* we found that after histone extraction the AMD-binding capacity of constitutive heterochromatin increases at the same rate or rather less than that of euchromatin. This again stresses the difference between constitutive and facultative heterochromatin. It further shows that besides the above discussed primary inactivity of constitutive heterochromatin there may also exist a repression mechanism due to histones comparable to that of facultative heterochromatin and euchromatin. The histones of constitutive heterochromatin may, however, have still other functions.

Histones have frequently been held responsible for condensation of chromatin (Littau *et al.*, 1965; Mirsky *et al.*, 1968; Richards and Pardon, 1970; Wagner, 1970; Miller *et al.*, 1971). This has often been thought to be connected with genetic inactivity. Our findings speak against a direct correlation between condensation and genetic inactivity: Constitutive heterochromatin is transcriptionally inactive, not only in the heteropycnotic but also in the isopycnotic (*i.e.*, non-condensed) state (Sieger *et al.*, 1970). Furthermore, the AMD-binding capacity is largely independent of the degree of chromatin condensation. The observation of Bolund (1970) that the heavily condensed chicken erythrocyte nuclei may bind even more AMD per unit DNA than nuclei of HeLa cells points in the same direction. Moreover, Kozlov and Georgiev (1970) did not find an increased RNA synthesis with destruction of the supercoil of the DNP complex. Finally it should be remembered that in many instances genetic inactivity is not necessarily connected with heteropycnosis (*e.g.*, Hotta and Stern, 1963; Konrad, 1963; Monesi, 1965; Henderson, 1964; also the findings on the inactivated mammalian X-chromosome—see Lyon for review, 1966, 1968). From these data it

follows that although condensation or heteropycnosis is quite often an accompanying phenomenon of genetically inactive chromatin, it is by no means the primary cause of genetic repression.

Acknowledgment. We wish to thank Mr. A.F. Gardner for helping with the translation of the manuscript. This paper was supported by the Deutsche Forschungsgemeinschaft.

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