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Marion Himes
CUNY Brooklyn College

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AN ANALYSIS OF HETEROCHROMATIN IN MAIZE ROOT TIPS

MARION HIMES

From the Department of Biology, Brooklyn College of The City University of New York, Brooklyn, New York 11210

ABSTRACT

The B chromosomes of maize are condensed in appearance during interphase and are relatively inert genetically; therefore they fulfill the definition of heterochromatin. This heterochromatin was studied in root meristem cells by radioautography following administration of tritiated thymidine and cytidine, and was found to behave in a characteristic way, i.e. it showed asynchronous DNA synthesis and very low, if any, RNA synthesis. A cytochemical comparison of normal maize nuclei with nuclei from isogenic maize stock containing approximately 15–20 B-chromosomes in addition to the normal complement has revealed the following: (*a*) the DNA and histone contents are greater in nuclei with B chromosomes; (*b*) the proportion of DNA to histone is identical with that of nuclei containing only normal chromosomes; (*c*) the amount of nonhistone protein in proportion to DNA in interphase is less in nuclei with B chromosomes than in normal nuclei. In condensed B chromosomes the ratio of nonhistone protein to DNA is similar to that in other condensed chromatin, such as metaphase chromosomes and degenerating nuclei. The B chromosomes appear to have no effect on nucleolar RNA and protein. Replication of B chromosomes is precisely controlled and is comparable to that of the ordinary chromosomes not only in synthesis for mitosis but also in formation of polyploid nuclei of root cap and protoxylem cells.

A consistent picture of heterochromatin has emerged in the last few years (7, 25). Several characteristics of heterochromatin have been described frequently enough to be included in a definition. These characteristics are as follows: (*a*) a condensed state in interphase; (*b*) genetic inertness; (*c*) late, asynchronous DNA replication; (*d*) low RNA synthesis in interphase. The concept of a differential, reversible state of condensation of a chromosome or part of a chromosome was first suggested by Cooper (8), who implied the control of gene activation by heterochromatization. The usefulness of this concept has been demonstrated by recent work on the X chromosome of female mammals (17) and the paternal chromosomes of male coccids (3, 7). The B chromosomes of maize have been shown to possess the first two characteristics of heterochromatin described above (23), and the latter two characteristics will be demonstrated here. In order to elucidate the nature of inactivated chromatin, this study of the B chromosomes was undertaken.

The number of B chromosomes found in nature is variable. High numbers of B chromsomes may be obtained with inbreeding and selection, since the disjunction of these chromsomes at meiosis is random (23). There is little phenotypic expression of their presence even when the number of B chromosomes exceeds that of ordinary chromosomes ($2N = 20$). In interphase nuclei with large numbers of B chromosomes, there are regions of dense chromatin near the nuclear periphery in addition to the characteristic nucleolar-associated chromatin (19). The appearance of this condensed chromatin is similar to that of the inactive chromatin described in other organisms. In this study

the interphase chromatin of normal maize nuclei is compared to that of maize nuclei containing approximately 20 B-chromosomes. Two methods of analysis are used: (*a*) cytochemical determinations of DNA, histone, and nonhistone protein, and (*b*) radioautography following incorporation of tritiated nucleic acid precursors. The present paper describes the histone and nonhistone protein content of interphase nuclei with B chromosomes. In addition, the effect of this heterochromatin on nucleolar RNA and protein is analyzed. Also examined is the replication of the B chromosomes in polyploid cells.

MATERIALS AND METHODS

Maize seeds (*Zea mays*) from a self-fertilized plant with 14 B-chromosomes and seeds from an isogenic stock without B chromosomes were used. Seeds from a stock with 25 B-chromosomes were also used.[1] After germination on wet filter paper until the roots were 4–8 mm in length, the root tips were removed and fixed either in 10% neutral formalin or by freeze-substitution (30), or they were placed in isotope for suitable periods of time and then removed and fixed similarly. The root tips were embedded in paraffin and sectioned at 4 or 20 μ. Sections of comparably treated normal root tips and B-chromosome-containing root tips were mounted together on the same slide for exact comparison.

Cytochemical Analysis

The Feulgen method of Stowell (27) was used for DNA determinations. Trichloroacetic acid was substituted for HCl in the hydrolysis for DNA–histone ratios. After photometric measurements of DNA content were made, the same slide was stained by the Alfert-Geschwind method for histone (2), and the same cells were remeasured for histone content.

The method for estimation of nonhistone protein by means of acid dyes at low pH (1.5–2.5) depends on the following rationale. When nucleic acids are removed from freeze-substituted material by hot trichloroacetic acid, the binding of acid dyes is increased. When the hot trichloroacetic acid is followed by dilute HCl extraction of histones, the amount of acid dye bound is approximately equal to the amount bound in the root tips that had neither the nucleic acids nor histone removed. These findings suggest that acid–dye binding by the amino groups of histones is suppressed by the presence of nucleic acids under these conditons of fixation.

Fast green was used, following nucleic acid removal, for estimation of nucleolar total protein, and naphthol yellow S was used following the Feulgen reaction, according to Deitch (9), to determine DNA:nonhistone protein ratios. Azure B was used for nucleolar RNA determinations according to Flax and Himes (10).

Photometric determinations of the amounts of dye binding were made with an apparatus similar to that described by Pollister and Moses (22). The light source was a tungsten 6 v ribbon filament stabilized by a storage battery; a Bausch and Lomb grating monochrometer was employed, with entrance and exit slits adjusted to 0.48 mm; a 16 mm objective served as a condenser; and a Zeiss 1.4 NA apochromat 90X oil-immersion objective was used. The single wavelength method was used for measuring light through thin sections of homogeneously stained regions of chromatin or nucleoli. The two wavelength method (21) was used for determining amounts of Feulgen stain and alkaline fast-green stain; the wavelengths chosen were 570 and 510 mμ for the former, and 610 and 572 mμ for the latter. For DNA determinations 20-μ thick sections were used; for DNA measurements followed by histone staining and measurements of dye bound, 4-μ thick sections were used to avoid any error in photometry owing to slight cytoplasmic staining. Precautions were taken to insure that the area measured of each nuclear section was precisely the same for the two determinations. The Feulgen–naphthol yellow S stain was measured through a small homogeneous area of chromatin at 570 and 435 mμ where the absorption curves of the two dyes do not overlap.

Radioautography

Tritiated thymidine (specific activity, 6.25 c/mmole) was diluted to 2–4 μc/ml in water; tritiated cytidine (specific activity, 1.21 c/mmole) was used at concentrations of 2–10 μc/ml; both were supplied to root tips for periods of 15 min–2 hr. Kodak AR 10 stripping film (Eastman Kodak Co., Rochester, N.Y.) was applied to mounted sections, following Feulgen staining, for thymidine-incorporation studies, and to unstained sections for cytidine-incorporation studies. RNase controls were used in conjunction with the latter. The films were exposed for 2–4 wk, developed, and stained with azure B (3 mg/ml, 30 sec).

RESULTS

DNA and Protein Composition of B Chromosomes

Table I indicates the relative amounts of DNA in the diploid and higher classes of maize nuclei.

[1] The author wishes to express appreciation to Professor M. M. Rhoades and Professor L. F. Randolph for providing the material used.

Although variations in DNA content among different strains of *Zea mays* are known (Swift, reference 28), the difference in the diploid amount of DNA in nuclei of the isogenic stocks studied here can be due to only the B chromosomes. No attempt was made to count the number of B chromosomes in each root tip. The elimination of roots with low numbers of B chromosomes was achieved by restricting the studies to root tips with large amounts of peripheral chromatin in interphase nuclei and with metaphase plates the diameter of which was almost double that of metaphase plates observed in normal maize. Variation in DNA content per nucleus among roots with different numbers of B chromosomes was expected. In order to avoid variations owing to DNA changes during the S period, only nuclei in telophase and metaphase were chosen. The DNA variation is similar in normal nuclei and B-chromosome-containing nuclei; this indicates that the B chromosomes behave exactly like normal ones with regard to the precise regulation of DNA synthesis and anaphase separation. Also, the amounts of DNA in nuclei of root cap and protoxylem cells indicate as exact a duplication of DNA in B chromosomes as in normal ones.

Table II *a* shows the relative amounts of staining by the Feulgen reaction and by alkaline fast

TABLE I

Relative Amounts of DNA (Feulgen Reaction)

Nuclear class	Meristem telophase or metaphase/2 2c	Protoxylem nuclei 4c	Protoxylem nuclei 8c	Root cap nuclei 4c	Root cap nuclei 8c
Normal maize	3.41 ± 0.08 (18)	7.4 (4)	13.1 (5)	6.7 (5)	12.1 (3)
B-chromosome-containing maize	4.95 ± 0.10 (12)	9.9 (4)	20.1 (3)	9.6 (4)	19.8 (4)
	4.25 ± 0.08 (10)				
	6.20 ± 0.11 (10)				

In all cases, the mean relative amount of Feulgen dye is given ± the standard error, with the number of different nuclei measured in parentheses.

TABLE II a

DNA–Histone Ratios (Feulgen–Fast Green, pH 8.1)

	Relative amount of Feulgen dye	Relative amount of fast green dye	Ratio
Normal maize, interphase	4.5 ± 0.2*	5.4 ± 0.1*	0.83
Normal maize, metaphase	4.8 ± 0.5	5.6 ± 0.3	0.87
B-chromosome-containing maize, interphase	4.4 ± 0.3	5.4 ± 0.3	0.82

* Mean ± standard error

TABLE II b

DNA–Nonhistone Protein Ratios (Feulgen–Naphthol Yellow S, pH 2.0)

	Meristem		Root cap
	Interphase	Metaphase	interphase
Normal maize	0.46 ± 0.01*	0.86 ± 0.03*	1.2*
B-chromosome-containing maize	0.62 ± 0.04	0.81 ± 0.01	1.7
B-chromosome-containing maize	0.70 ± 0.05	0.79 ± 0.03	0.9

* Mean ratios of extinction of dye at 570 mμ (Feulgen) to extinction of dye at 435 mμ (naphthol yellow S) ± standard error

green measured successively in the same nuclei. It can be seen that the ratio is the same in normal nuclei and in nuclei containing B chromosomes. Since many interphase cells are in the process of replication, it appears that DNA and histone are synthesized concurrently in the B chromosomes, as has been shown for normal chromosomes by Bloch and Godman (5).

When low pH acid–dye binding is compared to Feulgen stain in the same nucleus, it is apparent that there are significant differences in the ratios obtained for dispersed chromatin as compared to condensed chromatin (Table 11 *b*). The amount of dye bound at low pH, an indication of nonhistone protein, is greater in dispersed chromatin than in condensed chromatin as shown by the low ratio of Feulgen stain to naphthol yellow S stain (0.46). The condensed chromatin contains less nonhistone protein and shows higher ratios, i.e. 0.62–0.70 in nuclei with B chromosomes, 0.81 in metaphase chromosomes, and up to 1.7 in nuclei of root cap cells.

Nucleolar RNA and Protein

The effect of B chromosomes on the nucleolus is indicated in Table III, which shows comparisons of concentrations of azure B and fast green (low pH) bound to RNA and total protein, respectively. The nucleolar volumes are similar in the protoxylem cells in normal and isogenic B-chromosome-containing maize, and therefore the amounts of RNA and protein are the same. Nucleoli of meristem cells were not studied photometrically, but their diameters were measured to compare ranges of nucleolar volume throughout the cell cycle. There was a complete overlap of ranges, between 2.5 and 3.5 μ diameter for nucleoli in the normal and the B-chromosome-containing stocks. When material from a different source was examined, the nucleolar volume was significantly greater in nuclei with B chromosomes, but, since isogenic material with only the normal complement of chromosomes was not available, no further studies were carried out. It is possible that extra nucleolar-organizing chromatin was present, having been translocated either to a normal or a B chromosome.

That incorporation of tritiated cytidine into nucleoli of the isogenic stocks shows that the extra B-chromosomes have no measurable effect on RNA synthesis agrees well with the photometric determinations of amounts of RNA and protein. Fig. 1 shows a comparison of radioautographs of (*a*) a section through the meristem of a normal root tip and (*b*) a section through an isogenic, B-chromosome-containing root tip. The nucleoli that are cut at the surface show that the nucleolar volume and the number of grains in both sections are similar. This is also shown by grain counts after different times in the isotope (Table III).

Chromosomal DNA and RNA Synthesis

The incorporation of tritiated thymidine into meristem nuclei, followed by radioautography, indicates that the S period of the B chromosomes does not overlap to any considerable extent the S period of euchromatin. The peripheral location of the B chromosomes in interphase makes it possible

TABLE III

Nucleolar RNA and Protein Content and Cytidine Incorporation

	Normal maize	B-chromosome-containing maize
Nucleolar RNA and protein content		
Diameter (μ)	5.4 ± 0.3*	5.1 ± 0.4*
Azure B concentration‡	1.40 ± 0.03	1.32 ± 0.03
Fast green concentration‡ (pH 2.5, after removal of RNA)	0.79 ± 0.02	0.84 ± 0.02
Nucleolar RNA synthesis (grain counts per nucleolus after cytidine incorporation)		
15 min	1.3 ± 0.3*	1.1 ± 0.5*
30 min	7.4 ± 0.4	7.9 ± 0.3
1 hr	17.1 ± 0.3	15.0 ± 0.4

* Mean ± standard error

‡ Extinction in arbitrary units

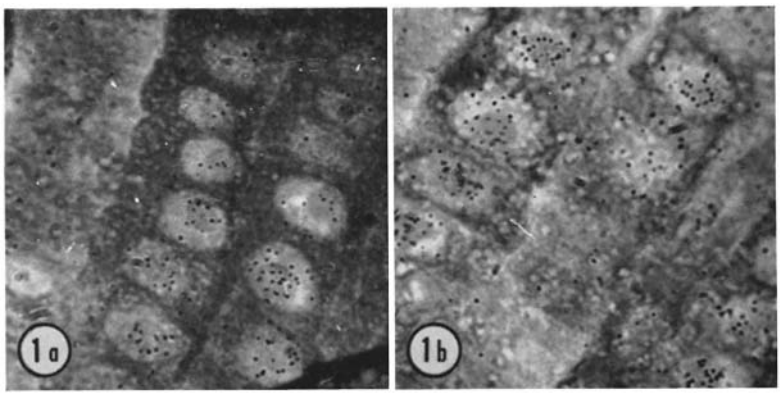

FIGURE 1 Radioautograph of maize meristem following 30 min incorporation of tritiated cytidine.

Fig. 1 *a*, maize with normal chromosome complement. Fig. 1 *b*, maize with approximately 15–20 B chromosomes. Note that labeling in nucleolar and nonnucleolar nuclear (chromatin) regions is similar in *a* and *b*.

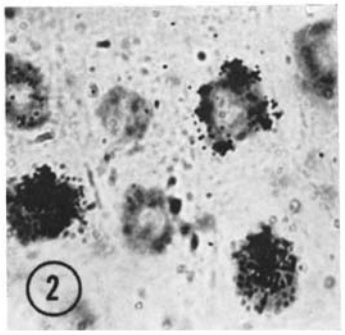

FIGURE 2 Radioautograph of maize with B chromosomes following 1 hr incorporation of tritiated thymidine. Note that nucleus at lower right has heavy label, perhaps exclusively over euchromatin. Nucleus at upper right shows label only over the heterochromatin.

to identify them in radioautographs. In many cases a heavy label appeared over all the chromatin except the peripheral clumps, while only rarely were the grains located exclusively over the peripheral regions, as in Fig. 2. This appearance is similar to that in other examples in which late replication of DNA in heterochromatin has been established (14). The infrequency of the appearance of such cells (about one in a thousand) compared to the frequency of appearance of labeled euchromatin and unlabeled heterochromatin indicates that the relative time spent in DNA synthesis is very much greater for euchromatin than for heterochromatin.

Chromosomal RNA synthesis was determined by counting grains developed over nonnucleolar areas of the nucleus after tritiated cytidine incorporation. The data presented in Table IV show no significant difference between grains over the nuclei with only the normal complement of chromosomes and those over the nuclei with additional B chromosomes. Since the nuclear volume is greater in nuclei with B chromosomes, the number of grains per unit area is less in B-chromosome–containing nuclei than in normal nuclei. It can be concluded, therefore, that the B chromosomes, which add at least DNA and histone to the interphase nucleus and increase the nuclear volume, do not contribute to the synthesis of chromosomal RNA. This agrees well with the idea that condensed chromatin is inactive in messenger-RNA synthesis (3).

DISCUSSION

The contribution of B chromosomes to interphase maize nuclei can be listed as follows: (*a*) the DNA and histone content are increased to different amounts in roots with different numbers of B chromosomes, as expected; (*b*) DNA and histone are maintained in the same proportion; (*c*) the increase in nonhistone protein is relatively low compared to the amounts present in euchromatin; (*d*) nucleolar RNA and protein remain unchanged; (*e*) there is little or no increase in chromosomal RNA synthesis.

The possibility that heterochromatin DNA and histone do not replicate in step with euchromatin DNA and histone during the multiple replications

TABLE IV

Cytidine Incorporation in Chromatin

	Grain counts per nucleus (over nonnucleolar areas)	
	30 min exposure	1 hr exposure
Normal maize	3.8 ± 0.4	8.5 ± 0.7
B-chromosome–containing maize	3.4 ± 0.5	8.8 ± 0.6

forming polyploid or polytene nuclei has been suggested by several workers. In the giant nurse cells of *Sciara coprophila* the L chromosomes that are present only in the germ line and are heterochromatic do not appear to replicate, while the other chromomes reach a DNA level which is about 1024 times the diploid amount of DNA (Himes and Crouse, reference 12). Also, on the basis of photometric determinations, Rudkin (26) has indicated that the heterochromatin of the Y chromosomes in *Drosophila* salivary gland cells does not replicate along with the other chromosomes. In addition, Nur (20), on the basis of purely morphological evidence, has stated that the heterochromatic paternal set of chromosomes does not replicate along with the euchromatic maternal set in the giant oenocytes of male coccids. In contrast to these three cases, the B chromosomes of maize appear to replicate in a regular fashion in the polyploid nuclei of the root cap and protoxylem cells. Although the B chromosomes are similar to the Y chromosome of *Drosophila*, the L chromosomes of *Sciara*, and the paternal set of chromosomes of male coccids with respect to late replication of DNA and low RNA synthesis, there appears to be a difference regarding the question of multiple replications. This may be correlated with the fact that the B chromosomes are not constant components maintained in the species as are the other chromosomes. It seems possible that the control of replication of heterochromatin has evolved in some organisms as an economy that prevents unnecessary syntheses, perhaps paralleling the evolution of chromosome diminution, which also eliminates the replication of unnecessary DNA in the somatic line. No such mechanism may have evolved in maize, in which the B chromosomes vary in number in nature and are usually absent

The fact that B chromosomes do not contribute to the amount of RNA and protein of the nucleolus is in contrast to the findings of Lin (15), who found a small increase in nucleolar RNA and protein in microsporocytes of maize containing B chromosomes. The reason for assuming that heterochromatin might affect the nucleolar RNA and protein content is that typically the nucleolar organizing region of the chromosomes is heterochromatin. The significance of this has yet to be explained. There is excellent evidence that the DNA coding for ribosomes is present at this location (24, 29) and that repeated segments of nucleolar organizer material are located at this site (18). Perhaps the heterochromatic condition of the nucleolar-associated DNA represents inactive sites that are repeated parts of the genome capable of coding for ribosomes. The heterochromatin of the B chromosomes may be qualitatively different, and so may not affect the nucleolus.

The same proportion of histone to DNA has been found in condensed chromatin and dispersed chromatin of maize in contrast to the finding of differences that have been reported for chromatin in coccids (4). The absence of increased histone in heterochromatin offers no support for the idea that histone regulates the amount of DNA-dependent RNA synthesis. The method used for determining histones, however, does not eliminate the possibility that histones could differ greatly qualitatively in the two conditions of chromatin and still maintain the same proportion of dye-binding sites. Other cytological methods have been used to distinguish histones in condensed chromatin and dispersed chromatin by Littau et al. (16) and by Himes (11) who obtained different results. At the present time, while the in vitro repression of DNA activity by histones is clear (6), any demonstration of histone involvement in heterochromatin inactivity is lacking.

The relative amount of nonhistone protein in the B chromosomes was found to be less than that of nonhistone protein in euchromatin in interphase maize nuclei. This characteristic of typical heterochromatin is shared by other condensed chromatin,

such as metaphase chromosomes. In two additional cases, the appearance of condensed chromatin has been characterized by loss of nonhistone protein: during pycnosis (1) and during spermatogenesis (13). Another characteristic of both heterochromatin and other condensed chromatin is the low RNA synthesis. The two phenomena are probably interrelated. The possibility that the nonhistone protein present in dispersed chromatin may be involved in strand separation of the DNA molecule that may be necessary for RNA synthesis is open to speculation.

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REFERENCES

1. ALFERT, M. 1958. *Exptl. Cell Res. Suppl. 6.* 227.
2. ALFERT, M., and I. I. GESCHWIND. 1953. *Proc. Natl. Acad. Sci. U.S.* **39**:991.
3. BERLOWITZ, L. 1965. *Proc. Natl. Acad. Sci. U.S.* **53**:68.
4. BERLOWITZ, L. 1965. *Proc. Natl. Acad. Sci. U.S.* **54**:476.
5. BLOCH, D. P., and G. C. GODMAN. 1955. *J. Biophys. Biochem. Cytol.* **1**:17.
6. BONNER, J., and R. C. HUANG. 1963. *J. Mol. Biol.* **6**:169.
7. BROWN, S. W., and U. NUR. 1964. *Science.* **145**: 130.
8. COOPER, K. 1959. *Chromosoma.* **10**:535.
9. DEITCH, A. 1955. *Lab. Invest.* **4**:324.
10. FLAX, M. H., and M. H. HIMES. 1952. *Physiol. Zool.* **25**:297.
11. HIMES, M. H. 1967. *J. Cell Biol.* **34**:77.
12. HIMES, M. H., and H. V. CROUSE. 1961. Abstracts of the First Annual Meeting of the American Society for Cell Biology. 88.
13. KAYE, J. S. and R. MCMASTER-KAYE. 1966. *J. Cell Biol.* **31**:159.
14. LIMA-DE-FARIA, A. 1959. *J. Biophys. Biochem. Cytol.* **6**:457.
15. LIN, M. 1955. *Chromosoma.* **7**:340.
16. LITTAU, V. C., V. G. ALLFREY, J. H. FRENSTER, and A. E. MIRSKY. 1964. *Proc. Natl. Acad. Sci. U.S.* **52**:93.
17. LYON, M. F. 1961. *Nature.* **190**:372.
18. MCCLINTOCK, B. *1934. Z. Zellforsch. Mikroskop. Anat.* **21**:294.
19. MORGAN, D. T. 1943. *J. Hered.* **34**:195.
20. NUR, U. 1966. *Chromosoma.* **19**:439.
21. ORNSTEIN, L. 1952. *Lab. Invest.* **1**:250.
22. POLLISTER, A. W., and M. J. MOSES. 1949. *J. Gen. Physiol.* **32**:567.
23. RANDOLPH, L. F. **1941**. *Genetics.* **26**:608.
24. RITOSSA, F. M., K. ATWOOD, and S. SPIEGELMAN. 1966. *Genetics.* **54**:819.
25. RUDKIN, G. T. 1965. *Proc. Intern. Congr. Genet. 11th The Hague 1963.* **2**:359.
26. RUDKIN, G. T. 1965. *Genetics.* **52**:470A.
27. STOWELL, R. E. 1945. *Stain Technol.* **20**:45.
28. SWIFT, H. H. 1950. *Proc. Natl. Acad. Sci. U.S.* **36**:643.
29. WALLACE, H., and M. L. BIRNSTIEL. 1966. *Biochim. Biophys. Acta.* **114**:296.
30. WOODS, P. S., and A. W. POLLISTER. 1955. *Stain Technol.* **30**:123.