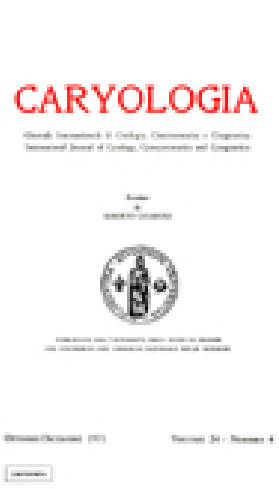


Caryologia: International Journal of Cytology, Cytosystematics and Cytogenetics

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tcar20

DNA/Histone Ratio in Different Regions of Polytene Chromosomes in the Embryo Suspensor Cells of Phaseolus Coccineus

J. L. Diez[a] & P. G. Cionini[a]

[a] Institute of Botany and Institute of Genetics, The University of Pisa, Pisa, Italy

Published online: 30 Jan 2014.

To cite this article: J. L. Diez & P. G. Cionini (1971) DNA/Histone Ratio in Different Regions of Polytene Chromosomes in the Embryo Suspensor Cells of Phaseolus Coccineus, Caryologia: International Journal of Cytology, Cytosystematics and Cytogenetics, 24:4, 463-470, DOI: 10.1080/00087114.1971.10796454

To link to this article: http://dx.doi.org/10.1080/00087114.1971.10796454

DNA/HISTONE RATIO IN DIFFERENT REGIONS OF POLYTENE CHROMOSOMES IN THE EMBRYO SUSPENSOR CELLS OF *PHASEOLUS COCCINEUS* *

J. L. DIEZ ** and P. G. CIONINI

Institute of Botany and Institute of Genetics, The University of Pisa, Pisa, Italy

Received: 17th February 1971

INTRODUCTION

There is no general agreement on DNA/histone ratio in cell nuclei. Many authors maintain that the DNA/histone combination is a specific one (MOORE 1959; WILKINS 1959) and that DNA/histone ratio remains constant in the tissues of a given species (RASCH and WOODARD 1959; DAS and ALFERT 1961). Several results demonstrate, however, that histone content, and/or histone reactivity to cytochemical techniques, change(s) in relation to tissue differentiation and/or cell functional stage (BLOCH and GODMAN 1955; GIFFORD and TEPPER 1962; PRITCHARD 1964*a, b*; BATTACHARIA *et al.* 1967; DWIVEDI and NAYLOR 1968; CORSI and AVANZI 1970).

The problem of histone constancy in relation to DNA structure and metabolic activity has been studied by SWIFT (1964) and GOROVSKY and WOODARD (1967) in giant chromosomes of *Drosophila virilis*. From the results obtained, they infer that no difference in histone content occurs between genetically active and inactive chromosomal loci. On the contrary, BERLOWITZ's results (1965) in testes of *Pseudococcus obscurus* point to « a quantitative difference in histones between euchromatin and heterochromatin which is genetically inactive and fails to metabolize RNA ». More recently RUCH and ROSSELET (1970) have reached similar conclusions in roots of *Rhoeo discolor*.

Polytene chromosomes occur in the embryo suspensor cells of *Phaseolus* (NAGL 1962*a, b*, 1967, 1969*a, b*, 1970; AVANZI *et al.* 1970). They show

---

* Publication no. 49 from the Laboratorio di Mutagenesi e Differenziamento, C.N.R., Pisa, Italy.

** Post-doctoral fellowship from interchange between C.S.I.C. (Spain) and C.N.R. (Italy). Present address: Max-Planck-Institut für Biologie, Tübingen, Germany.

large, seemingly heterochromatic knobs, which AVANZI *et al.* have regarded as chromosomal regions capable of extra DNA synthesis and comparable to the « DNA puffs » (PAVAN 1965) of several Sciarid flies. AVANZI *et al.* have also shown that these regions may be either inactive or active in incorporating ^{3}H-thymidine and ^{3}H-uridine, as is the case with the DNA body of *Acheta domesticus* (LIMA DE FARIA *et al.* 1968). As in *Acheta* (LIMA DE FARIA *et al.* 1968), after Feulgen staining, the seemingly heterochromatic regions of *Phaseolus* may or may not show an outer puffed shell (NAGL 1970; AVANZI *et al.* 1970).

The aim of the present work is to investigate the DNA/histone ratio in these segments of the polytene chromosomes of *Phaseolus coccineus* suspensor cells in relation to their structure.

MATERIAL AND METHODS

Seeds from plants grown in the open air in the Pisa Botanical Garden were fixed in 10% neutral formalin for 3 hours, washed overnight in tap water, embedded in paraffin and sectioned at 10 μm. Sections were stained by the Feulgen method, substituting 1N trichloroacetic acid for 1N HCl in the hydrolysis and in the Schiff reagent. Slides were afterwards washed three times (10' every time) in SO_2 water and mounted in Canada balsam. After mapping of several chromosome segments in microphotographs, each segment was scanned for Feulgen-DNA by means of a Deeley type cytophotometer produced by Barr and Stroud, Glasgow (integrating microdensitometer type GN2), following the procedure of MCLEISH and SUNDERLAND (1961). Slides were then dismounted in xilene and DNA was extracted with trichloroacetic acid 5% at 90°C for 15'. The same segments were thereafter scanned, with the same equipment, for amount of alkaline fast green stainable histone, according to the method of ALFERT and GESCHWIND (1953). Since measurements were made on sections, chromosome regions were often well isolated from each other. When this was not the case, measurement of Feulgen and fast green absorption was made by isolating the same area by means of an identical diaphragm aperture.

RESULTS AND DISCUSSION

In Fig. 1, DNA/histone ratios in different chromosome regions are presented. Seemingly heterochromatic regions were divided into two categories according to their appearance in Feulgen stained material: i) ' active ' chromocenters (regions which appeared peripherally decondensed, with a shell of thin loops resembling a lampbrush state (NAGL 1970; AVANZI *et al.* 1970): Fig. 2, chromocenter on the left) and ii) ' inactive ' chromocenters (regions in a condensed state, devoid of loops: Fig. 2, chromocenter on the right). The figure shows a large variability in Feulgen-DNA/histone-fast

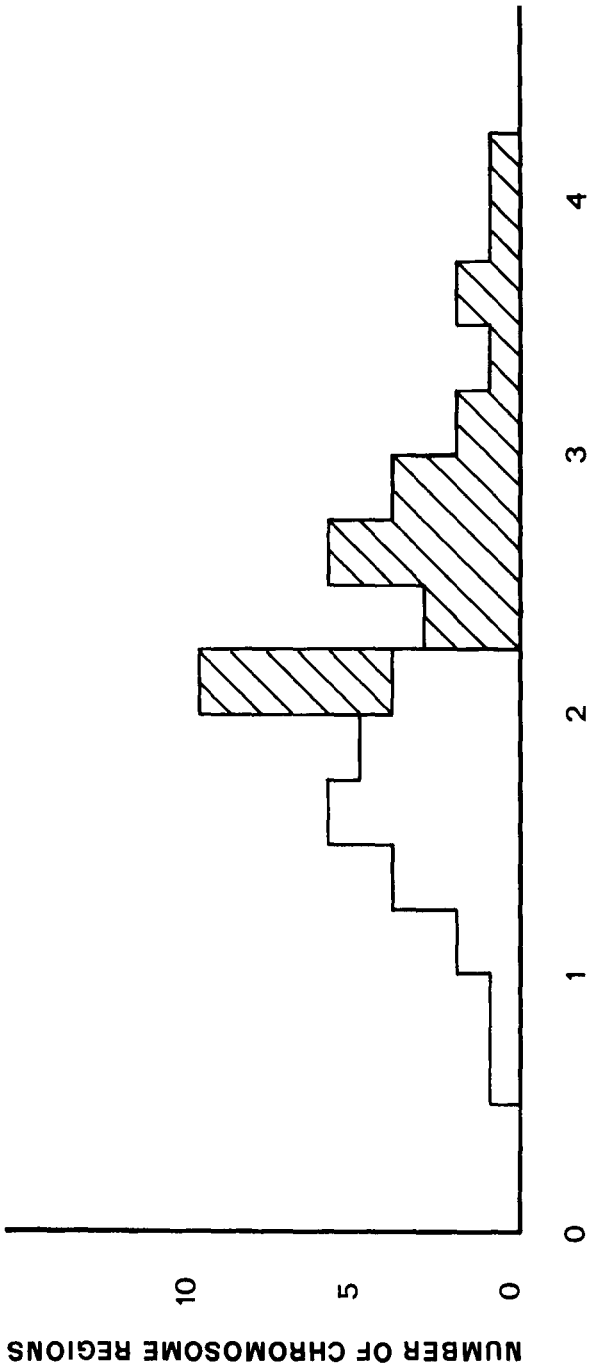
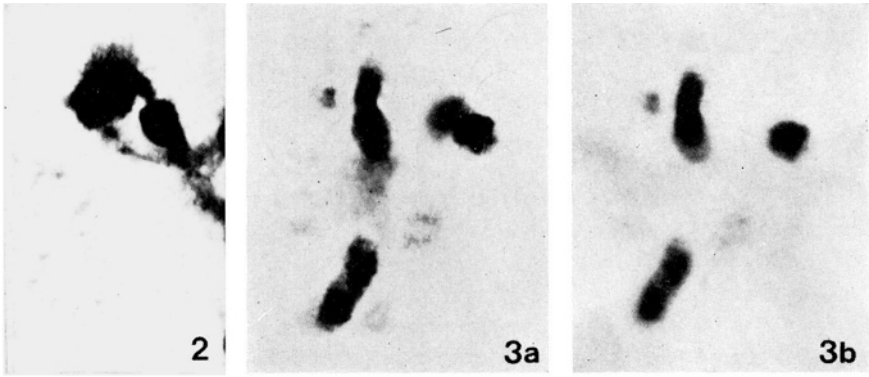


Fig. 1. — Feulgen-DNA/histone-fast green ratios of different polytene chromosome regions of *Phaseolus coccineus* embryo suspensor cells. Open bars: 'inactive' chromocenters; dashed bars: 'active' chromocenters.

green ratios. It is apparent that lower ratios (greater histone content) occur in the ' inactive ' regions, whilst higher ratios (lower histone content) occur in the ' active ' regions. That DNA in puffed or lampbrush state has much less (or has not at all) histone content than condensed DNA, is clear comparing Figs. 3a and 3b. Fig. 3a shows some chromosome regions after Feulgen staining, Fig. 3b shows the same regions after DNA extraction and fast green staining of histones: the areas stained by Feulgen are larger than areas stained by fast green.



Figs. 2, 3a, 3b. — Embryo suspensor cells of *Phaseolus coccineus*. Sections 10 μm. x 1000.
Fig. 2. — Two seemingly heterochromatic DNA knobs with different peripheral structure after Feulgen staining: i) with a shell of thin loops resembling a lampbrush state (' active ' chromocenter, on the left) and ii) condensed and devoid of loops (' inactive ' chromocenter, on the right).
Figs. 3a and 3b. — The same chromosome regions stained first with Feulgen (3a) and afterwards with fast green at pH 8.1 after DNA extraction (3b). Note the lack of fast green staining on some portions.

The lowering of fast green stainability, and hence differences in Feulgen/fast green ratios, may imply, of course, not only a decrease in histone content, but also some histone changes such as acetylation, methylation or phosphorylation. BLOCH and HEW (1960) have shown that acetylation of lysine ε-N amino groups results in a modification of fast green stainability of histone. The possibility that DNA/histone ratio, as revealed by Feulgen/fast green ratio, might be influenced by other circumstances can not be excluded, however (SINGER 1952; ALLFREY *et al.* 1968).

If it is assumed that our results indicate variations in histone content, it is apparent that these variations, as expressed through the DNA/histone ratio, are strictly related to the structural condition of DNA.

As pointed out by AVANZI *et al.* (1970), the seemingly heterochromatic knobs in *Phaseolus* suspensor cells may be the result of a localized extra DNA synthesis. That extra DNA is complexed with histones is known (LIMA

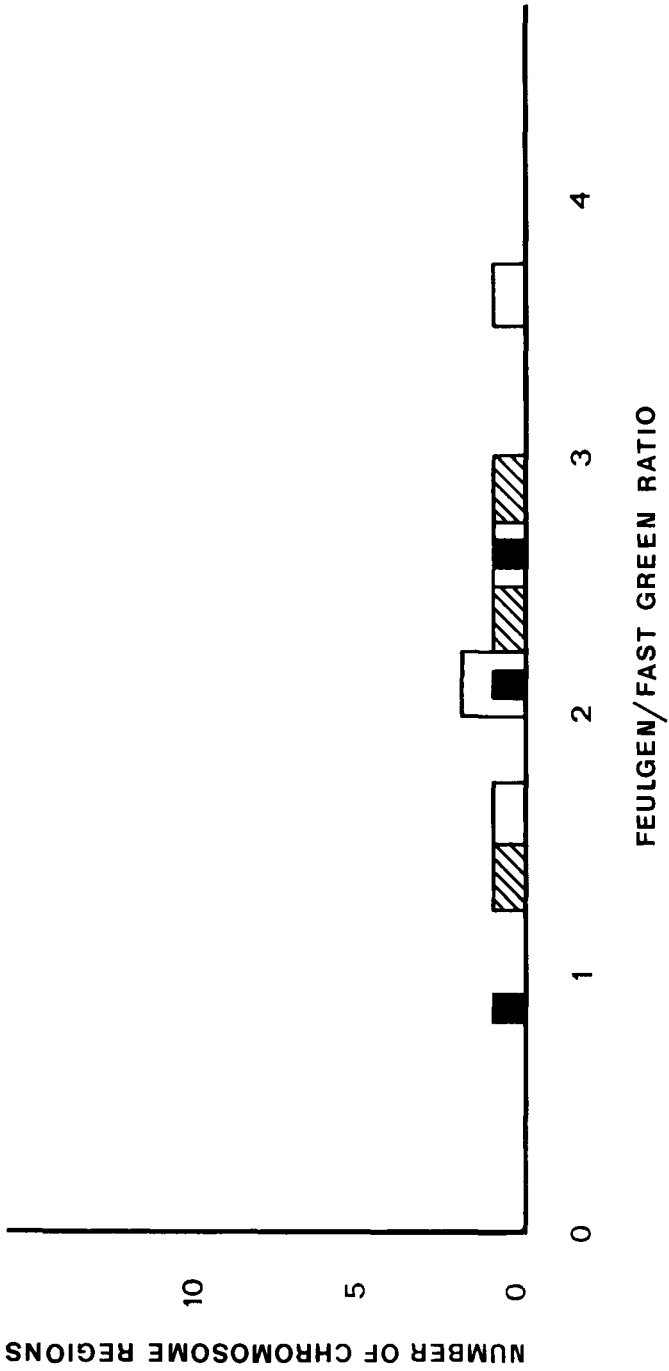


Fig. 4. — Feulgen-DNA/histone-fast green ratios of different polytene chromosome regions in a single embryo suspensor cell of *Phaseolus coccineus*. Dashed bars: chromocenters belonging to one and the same chromosome; solid bars: nucleolar organizers.

DE FARIA and MOSES 1966; LIMA DE FARIA *et al.* 1968; KATO 1968; AVANZI *et al.* 1970). The labelling experiments of AVANZI *et al.* (1970) indicate that incorporation of tritiated precursors of nucleic acids (as possible *in situ* metabolic activity of extra DNA) is present in several but not in all chromocenters. This observation finds confirmation in our results, which seem to indicate that not all extra DNA is complexed, or equally complexed, with histone and, consequently, equally active in trascription. In fact, evidence has been accumulated that association of histones with DNA can suppress both DNA-dependent RNA synthesis and DNA activity as primer for its own duplication (cf. VENDRELY and VENDRELY 1966).

Some possible differences in DNA-histone complexes are indicated by the fact that, in segments with higher DNA/histone ratio (' active ' chromocenters), the fast green stained area is smaller than the Feulgen stained area in the same chromosome region. This demonstrates the existence of a peripheral portion of DNA not complexed (or very weakly complexed) with histone (Figs. 3a and 3b). Therefore, the correlation between DNA structure and histone content goes in the expected sense. In this connection, it is relevant that addition of histones to lampbrush chromosomes causes the same effect as addition of actinomycin D, *i.e.* retraction of DNA loops and discontinuance of RNA synthesis (ALLFREY and MIRSKY 1964).

Different Feulgen-DNA/histone-fast green ratios occur not only in different regions of the chromosomes in the same cell, but also in different segments of one and the same chromosome. This situation is apparent in Fig. 4, in which the chromocenters of one and the same cell are considered. Fig. 4 also shows that different nucleolar organizers in the same nucleolus (*Phaseolus coccineus* suspensor cells generally show an unique large nucleolus) may have different DNA/histone ratios. These findings are not surprising, however. AVANZI *et al.* (1970) have even reported some examples of functional asynchrony within a chromosome region. Our data are not enough detailed to confirm, at the level of DNA/histone ratio, their finding.

Acknowledgements. — The authors are grateful to Prof. SILVANA AVANZI for help and discussion during the course of tne work and to Prof. F. D'AMATO for critical reading of the manuscript. They also acknowledge the very efficient technical assistance of Mrs. AMELIA PARTEARROYO.

REFERENCES

ALFERT M. and GESCHWIND I. I., 1953. — *A selective staining method for the basic proteins of cell nuclei.* Proc. Natl. Acad. Sci., 29: 991-999.

ALLFREY V. G. and MIRSKY A. E., 1964. — *Role of histone in nuclear function.* In: *The nucleohistone*, J. Bonner and P. Ts'o (ed.), Holden-Day Inc., San Francisco, London, Amsterdam, pp. 267-288.

ALLFREY V. G., POGO B. G. T., LITTAU V. C., GERSHEY E. L. and MIRSKY A. E., 1968. — *Histone acetylation in insect chromosomes.* Science, 159: 314-316.

AVANZI S., CIONINI P. G. and D'AMATO F., 1970. — *Cytochemical and autoradiographic analy ses on the embryo suspensor cells of Phaseolus coccineus.* Caryologia, 23: 605-638.

BERLOWITZ L., 1965. — *Analysis of histone in situ in developmentally inactivated chromatin.* Proc. Natl. Acad. Sci., 54: 476-480.

BHATTACHARIA P. K., SHAW M. and NAYLOR J. M., 1967. — *Nucleic acid and protein changes in wheat leaf nuclei during rust infection.* Science, 150: 1605-1607.

BLOCH D. P. and HEW J. C., 1960. — *Changes in nuclear histones during fertilization and early embryonic development in the pulmunate snail Helix aspersa.* J. Bioph. Bioch. Cytol., 8: 69-81.

BLOCH D. P. and GODMAN G. C., 1955. — *A microphotometric study of the synthesis of desoxy-ribonucleic acid and nuclear histone.* J. Bioph. Bioch. Cytol., 1: 17-28.

CORSI G. and AVANZI S., 1970. — *Cytochemical analyses on cellular differentiation in the root tip of Allium cepa.* Caryologia, 23: 381-394.

DAS N. K. and ALFERT M., 1961. — *Accelerated DNA synthesis in onion root meristem during X-irradiation.* Proc. Natl. Acad. Sci., 47: 1-6.

DWIVEDI R. S. and NAYLOR R. M., 1968. — *Influence of apical dominance on the nuclear pro-teins in cells of the lateral bud meristem in Tradescantia paludosa.* Canadian J. Bot., 46: 289-298.

GIFFORD E. M. and TEPPER H. B., 1962. — *Histochemical and autoradiographic studies of floral induction in Chenopodium album.* Amer. J. Bot., 49: 706-714.

GOROVSKY M. A. and WOODARD J., 1967. — *Histone content of chromosomal loci active and inactive in RNA synthesis.* J. Cell Biol., 33: 723-728.

KATO K., 1968. — *Cytochemistry and fine structure of elimination chromatin in Dytiscidae.* Exptl. Cell Res., 52: 507-522.

LIMA DE FARIA A. and MOSES M. J., 1966. — *Ultrastructure and cytochemistry of metabolic DNA in Tipula.* J. Cell Biol., 30: 177-192.

LIMA DE FARIA A., NILSSON B., CAVE D., PUGA A. and JAWORSKA H., 1968. — *Tritium labelling and cytochemistry of extra DNA in Acheta.* Chromosoma, 25: 1-20.

McLEISH J. and SUNDERLAND N., 1961. — *Measurements of deoxyribosenucleic acid (DNA) in higher plants by Feulgen photometry and chemical methods.* Exptl. Cell Res., 24: 527-540.

MOORE S., 1959. — *On the distribution of histones.* In: *Nucleoproteins,* R, Stoops (ed.), Solvay Inst., 11th Chem. Conf., Interscience Publ. Inc., New York, pp. 77-103.

NAGL W., 1962*a*. — *Über Endopolyploidie, Restitutionskernbildung und Kernstrukturen im Suspensor von Angiospermen und einer Gymnosperme.* Österr. Bot. Z., 109: 431-494.

—, 1962*b*. — *4096-Ploidie und 'Riesenchromosomen' im Suspensor von Phaseolus coccineus.* Naturwiss., 49: 261-262.

—, 1967. — *Die Riesenchromosomen von Phaseolus coccineus L. Baueigentümlichkeiten. Struk-turmodifikationen, Zusätzliche Nukleolen und Vergleich mit den mitotischen Chromosomen.* Öster. Bot. Z., 114: 171-182.

—, 1969*a*. — *Banded polytene chromosomes in the legume Phaseolus vulgaris.* Nature, 221: 70-71.

—, 1969*b*. — *Puffing of polytene chromosomes in a plant (Phaseolus vulgaris).* Naturwiss., 56: 221-222.

—, 1970. — *Temperature-dependent functional structures in the polytene chromosomes of Pha-seolus, with special reference to the nucleolus organizers.* J. Cell Sci., 6: 87-107.

PAVAN C., 1965. — *Nucleic acid metabolism in polytene chromosomes and the problem of dif-ferentiation.* Brookhaven Symp. in Biology, 18: 222-241.

PRITCHARD H. N. 1964*a*. — *A cytochemical study of embryo sac development in Stellaria media.* Amer. J. Bot., 51: 371-378.

—, 1964*b*. — *A cytochemical study of embryo development in Stellaria media.* Amer. J. Bot., 51: 472-479.

RASCH E. and WOODARD J. W., 1959. — *Basic proteins of plant nuclei during normal and pathological cell growth.* J. Bioph. Bioch. Cytol., 6: 263-276.

RUCH F. and ROSSELET A., 1970. — *A cytological study of euchromatin and heterochromatin in roots of Rhoeo discolor.* Exptl. Cell Res., 62: 219-227.

SINGER M., 1952. — *Factors which control the staining of tissue section with acid and basic dyes.* Int. Rev. Cytol., 1: 211-255.

SWIFT H., 1964. — *The histones of polytene chromosomes.* In: *The nucleohistone*, J. Bonner and P. Ts'o (ed.), Holden-Day Inc., San Francisco, London, Amsterdam, pp. 169-183.

VENDRELY R. and VENDRELY C., 1966. — *Biochemistry of histones and protamines.* Protoplasmatologia, 5, 3c: 1-88.

WILKINS M. H. F., 1959. — *Molecular structure of deoxyribonucleoproteins.* In: *Nucleoproteins*, R. Stoops (ed.), Solvay Inst., 11th Chem. Conf., Interscience Publ. Inc., New York, pp. 45-76.

SUMMARY

The DNA/histone ratio was calculated through Feulgen/fast green absorption in different regions of polytene chromosomes in *Phaseolus coccineus* embryo suspensor cells. A great variability of ratios, related with the structural characteristics of DNA in the different regions, has been found. This seems to indicate that complexes of histone with DNA may depend on changes in DNA metabolic activity. In one and the same cell, and even in one and the same chromosome, different chromosome segments have different DNA/histone ratios.

These findings are discussed in relation to some characteristics of polytene chromosomes in *Phaseolus* suspensor cells.