

Size Correlations Between Heterochromatin and Nucleolus During Interphase in the Quail, *Coturnix coturnix japonica*

Heterochromatin association to nucleolus is a well-known phenomenon^{1,2}. Electron microscopy shows connections between perinucleolar heterochromatin and intranucleolar fibres³, but, at the present time, the function of this fraction of heterochromatin is controversial.

Most authors think that constitutive heterochromatin is genetically inactive. Incorporation of ³H-uridine in lymphocytes nuclei has brought FRENSTER⁴ to the conclusion that heterochromatin is not responsible for the elaboration of ribosomal RNA. YUNIS and YASMINEH⁵ insist on the structural and protective role of this part of the genome, and WALKER⁶ concludes that satellite DNA, which in part seems to correspond to constitutive heterochromatin, is not involved in transcription or protein synthesis.

On the other hand, LIMA-DE-FARIA⁷ has reported amplification of ribosomal cistrons in oocytes of *Acheta*, which takes the aspect of a large heterochromatic mass, called DNA-body, and which is surrounded by nucleolar material. The observation of KINSKY and PERA⁸, who found that in the proximal tubules of renal cells there is no chromocentre but a large nucleolus when, in distal tubules, there are large chromocentres and a small nucleolus, is in favour of a direct relationship between constitutive heterochromatin and nucleolar organization.

We report here similar observations which show inverse correlation between the size of the heterochromatin and the nucleolus in the fibroblasts of the Quail (*Coturnix coturnix japonica*).

Tissue fragments of 8-day-old quail embryos were explanted on coverslips in Leighton tubes and fed with Hanks tissue culture medium enriched with fetal serum and chicken embryonic extract (15:7:5). After 6 days, some droplets of chicken embryonic extract are added and the next day the preparations are fixed in toto in Carnoy. The slides were stained with acridine orange (AO) following this procedure: after progressive rehydration in ethanol baths and a step in Mc-Uvaine's buffer,

PH = 6, the slides were stained with AO (0.125 mg/ml of buffer) for 5 min, rinsed and mounted in the same buffer. Observations were made with Zeiss photomicroscope II equipped with an HBO-200 mercury lamp, using a BG-12 excitation filter and a No. 50 barrier filter.

In the fibroblasts examined, we generally noted a large nucleolus stained in red by AO. In some nuclei, there are more than one nucleolus, but always smaller than the main one. All these nucleoli, which are more or less defined, are always associated to heterochromatic masses stained in green-yellow by AO. Indeed, next to each nucleolus, chromatin aggregates which differ in size, number and appearance are observed. In some cells, only one large chromocentre could be observed while in other cells, several smaller ones exist. They could be separated from each other, or joined, forming a circular body around the nucleolus which is more or less dense. Most interesting is the constant inverse relation between the size of the nucleoli and the heterochromatin adjacent to them. Figure 1 shows fibroblasts nuclei with large nucleoli and small chromocentres, while in Figure 2 the inverse is observed. Some largest nucleoli are deprived of any visible heterochromatin.

¹ T. CASPERSSON, *Cell Growth and Cell Function* (Norton, New-York 1950), p. 185.

² A. LIMA-DE-FARIA and J. REITALU, *Symp. int. Soc. Cell Biol.* 3, 249 (1964).

³ J. G. LAFONTAINE and A. LORD, in *Handbook of Molecular Cytology* (Ed. A. LIMA-DE-FARIA; American Elsevier, New-York 1969), p. 382.

⁴ J. H. FRENSTER, in *Handbook of Molecular Cytology* (Ed. A. LIMA-DE-FARIA; American Elsevier, New-York 1969), p. 251.

⁵ J. Y. YUNIS and W. G. YASMINEH, *Science* 174, 1200 (1971).

⁶ P. M. B. WALKER, *Nature, Lond.* 229, 306 (1971).

⁷ A. LIMA-DE-FARIA in *Handbook of Molecular Cytology* (Ed. A. LIMA-DE-FARIA; American Elsevier, New-York (1969), p. 278.

⁸ I. KINSKY and F. PERA, *Verh. anat. Ges.* 67, Versamml., Köln (1972).

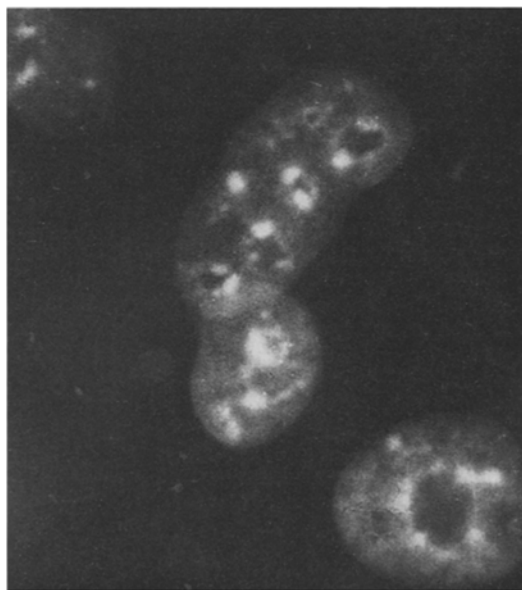


Fig. 1. Quail's nuclei fibroblasts stained with acridine orange. Small chromocentres associated to abundant nucleolar material.

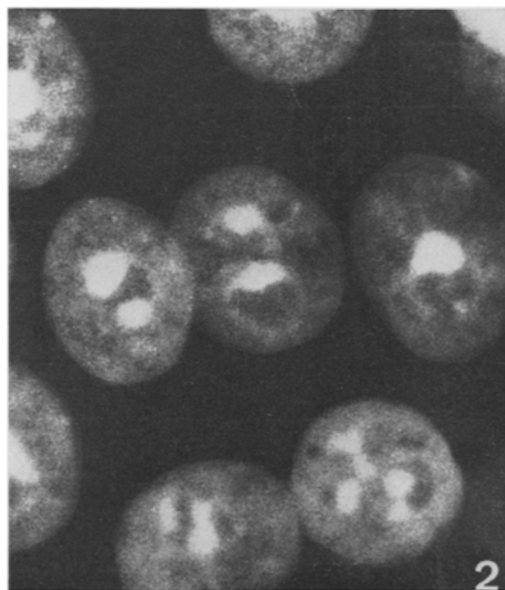


Fig. 2. Larger chromocentres adjacent to reduced nucleoli.

LE DOUARIN⁹ has observed that, in the quail, the dense chromatin aggregates in interphase are associated to nucleolar RNA. COMINGS and MATTOCCIA^{10,11} have shown that in this bird the microchromosomes are nucleolar organizers. Moreover, they are heterochromatic and contain the heavy shoulder DNA. We can assume that the chromocentres associated to nucleoli in the quail correspond to microchromosomes which are more or less heteropycnotic in the interphase cells. It is well known that nucleoli are prominent in cells which are rich in RNA and active in protein synthesis^{1,7}. On the contrary, small nucleoli are found in non-active cells. In *Microtus agrestis* constitutive heterochromatin despiralizes by TSH stimulation in thyroid cells¹².

All these observations, together with our work, suggest that the despiralization of heterochromatin is responsible for the apparition of active nucleolar material, complete despiralization giving birth to the largest nucleoli with the disappearance of any observable chromocentres. When only a fraction of heterochromatin is despiralized, a large and dense chromocentre is then associated to a very small nucleolus, as reported here. This interpretation is in accordance with all the aspects observed in our preparations where we have compared the size of the nucleoli and the heterochromatic masses adjacent to them.

Résumé. L'étude des noyaux des fibroblastes de Caille cultivés in vitro a été réalisée à l'aide de l'orange d'acridine. L'emploi de ce fluorochrome permet une distinction aisée du nucléole et de l'hétérochromatine qui lui est associée. On observe une corrélation inverse entre le volume du nucléole et celui de l'hétérochromatine.

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⁹ N. LE DOUARIN, *Annls Embryol. Morphogen.* 4, 125 (1971).

¹⁰ D. E. COMINGS and E. MATTOCCIA, *Chromosoma* 30, 202 (1970).

¹¹ D. E. COMINGS and E. MATTOCCIA, *Expl Cell Res.* 70, 256 (1971).

¹² E. SCHNEIDER, U. HEUKAMP and F. PERA, *Chromosoma* 41, 167 (1973).

¹³ The technical assistance of Mrs. C. FOUET and Miss N. LEGUILLOU is gratefully acknowledged.

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Comportement des mastocytes chez les rats traités au perchlorate de potassium

Le rôle important joué par les mastocytes dans les tissus est dû à leur synthèse en composants bioactifs tels que l'héparine, l'histamine et la sérotonine¹. De nombreux travaux ont été consacrés à ces cellules, à leurs produits de synthèse², à leurs modifications provoquées par différents agents cytotoxiques¹ et à leur dégranulation en rapport avec le métabolisme du collagène³.

Le perchlorate de potassium administré par voie buccale agit comme un antithyroïdien en bloquant la

synthèse des hormones thyroïdiennes. La littérature à ce sujet a été revue précédemment⁴. Contrairement aux autres antithyroïdiens, il semble que le perchlorate ait en outre une action directe sur le métabolisme de la thyroxine au niveau tissulaire⁵. Il nous a donc paru intéressant d'étudier la distribution des mastocytes et leurs modifications chez des rats traités par cet antithyroïdien.

Pour cette expérience, on utilise des rats Wistar mâles de 7 semaines de 150–200 g; 8 rats servent de contrôle et 8 autres reçoivent 0,45 g/kg de poids par jour de perchlorate de potassium. Les déterminations quantitatives (mastocytes par mm²) et qualitatives (formes dégranulées) se font dans la peau (région dorsale), la thyroïde, le foie et le poumon au moyen des colorations suivantes: Bleu Alcian-Safranine⁶ et Bleu Alcian-PAS. Dans les coupes de peau, les mastocytes sont divisés en 3 groupes: groupe I (mastocytes dont le cytoplasme est rempli de granules denses, sans aucun signe de dégranulation); groupe II (mastocytes présentant quelques signes de dégranulation reconnaissables à la présence de quelques granules en dehors du cytoplasme); groupe III (mastocytes fortement dégranulés et restes de cytoplasme contenant des granules⁷). Dans la thyroïde, les mastocytes sont divisés en mastocytes sans signe de dégranulation et mastocytes présentant des signes de dégranulation plus ou moins accentués. Les résultats sont analysés au moyen du test *t* et de l'erreur standard.

Tableau I. a) Pourcentage des différents types de mastocytes rencontrés dans le derme

Groupe I ^a		Groupe II		Groupe III ^b	
contrôles	traités	contrôles	traités	contrôles	traités
6 rats	6 rats	6 rats	6 rats	6 rats	6 rats
35,1 ± 2,1	28,4 ± 3,9	33,8	33,9	30,8 ± 2,4	37,4 ± 3,7

Pour chaque animal, on compte 1000 mastocytes; les pourcentages sont effectués à partir de cette valeur.

^a 0,05 > *p* < 0,01.

^b 0,02 > *p* > 0,01.

b) Nombre de mastocytes par mm² de derme cutané

contrôles 6 rats	traités 6 rats
15,9 ± 1,1	12,8 ± 1,9

Pour chaque animal, on compte le nombre total de mastocytes sur une surface de 40,56 mm². Les résultats sont exprimés en nombre de mastocytes par mm².

Sont reportées dans ce tableau les valeurs moyennes et les déviations standards.

¹ H. SELYE, *The Mast Cells* (Butterworths, Washington 1965).

² J. W. COMBS, *J. Cell Biol.* 37, 563 (1966).

³ W. RAAB, *Z. Immunitätsforsch.* 133, 180 (1967).

⁴ A. SPRECA, M. LASZLO et J. P. MUSY, *Acta pharm. Helv.* 48, 297 (1973).

⁵ T. YAMADA, *Endocrinology* 81, 1285 (1967).

⁶ S. S. SPICER, *Am. J. Path.* 37, 457 (1960).

⁷ A. SPRECA, L. MODIS et G. CONTI, *J. Embryol. exp. Morph.* 26, 459 (1971).