Atti Accademia Nazionale dei Lincei

Classe Scienze Fisiche Matematiche Naturali

Rendiconti

Giorgio Prantera, Mirella Di Castro, Enzo Marchetti, Angela Rocchi

^{3}H-Actinomycin-D labelling pattern of Chinese hamster chromosomes

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Serie 8, Vol. **60** (1976), n.3, p. 315–319.

Accademia Nazionale dei Lincei

<http://www.bdim.eu/item?id=RLINA_1976_8_60_3_315_0>

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Accademia Nazionale dei Lincei, 1976.

Citogenetica. — ^{3}H-*Actinomycin*-D *labelling pattern of Chinese hamster chromosomes.* Nota di GIORGIO PRANTERA, MIRELLA DI CASTRO, ENZO MARCHETTI e ANGELA ROCCHI, presentata (*) dal Socio G. MONTALENTI.

RIASSUNTO. — Cellule fissate della linea C–125 di Hamster cinese sono state trattate con actinomicina–D–H^3, questo antibiotico si lega al DNA in presenza di basi G–C. Lo studio della distribuzione della marcatura su tre cromosomi morfologicamente riconoscibili ha portato ad identificare la presenza di zone cromosomiche di costante maggiore o minore intensità di marcatura e di zone solo casualmente marcate, ma non di zone costantemente prive di marcatura.

Il pattern di marcatura da actinomicina–D–H^3 è stato confrontato con il pattern da despiralizzazione ottenuto, sugli stessi cromosomi, con l'uso di Hoechst 33258, un fluorocromo che agisce su aree cromosomiche ricche in basi A–T. Questo confronto non ha rivelato la presenza di zone di marcatura complementari alle zone di despiralizzazione.

INTRODUCTION

A chromosome labelling pattern has been obtained by research workers using tritiated actinomycin-D (^{3}H-AMD) on the fixed chromosomes of a number of plants [1, 6, 5] and of man [14, 21].

The binding capacity of actinomycin-D with DNA is highly selective and it has been shown that it binds preferentially with double stranded DNA and only in the presence of guanine, intercalating itself between the guanine-cytosine sequences and forming specific hydrogen bonds with guanine [19, 15, 24]. In a previous study [22] cultures of Chinese hamster cells were supplied with Hoechst 33258, a benzimidazole derivative fluorochrome. This compound has caused the appearance of non condensed areas in a constant position on hamster metaphase chromosomes, a phenomenon which has also appeared, in different degrees of intensity, in mouse chromosomes [12], in those of *Drosophila melanogaster* [18] and of man (Pimpinelli *et al.* submitted for publication) after the same treatment. The decondensed chromosomal segments should contain AT-rich DNA [25, 7]. Chinese hamster DNA, unlike that of the other organisms mentioned, when spun in CsCl density gradient did not, however, show any satellite bands particularly rich in AT bases, while it did show a small shoulder heavier than the main band which represents about 4 % of the DNA of the whole nuclei [9]. According to the data of Evenson *et al.* (1972) who partially denatured Chinese hamster DNA, it is likely that this shows short interspersed sequences rich in AT bases.

As a result it has seemed to be of interest to study the distribution of ^{3}H-AMD labelling on the chromosomes of a Chinese hamster cell line.

(*) Nella seduta del 13 marzo 1976.

Materials and methods

The Chinese hamster cell line C-125 described by Olivieri *et al.* (1971) and Palitti *et al.* (1974) was used.

Preparations were obtained with the normal air-drying technique and fixed with methanol: acetic acid, 3 : 1. The slides used were previously treated with cold AMD (10 γ/ml) in order to reduce the autoradiographic background.

All slides were covered with a solution of ^{3}H-AMD (Schwarz Bio. Res. spec. act. 8.4Ci/mM, conc. 4.15Ci/ml) for 30 minutes and washed in running water for many hours. Kodak NTB-2 liquid emulsion was used for the autoradiography. The slides were developed after exposure times varying from 2 to 9 days and stained with Mayer's haematoxylin. After having been photographed the preparations had the films removed [3] and were rephotographed.

Results and discussion

There are not very many chromosomes of the Chinese hamster cell line C-125 recognizable without the banding technique. Amongst these we have had to discard also those which, being practically metacentric, did not permit

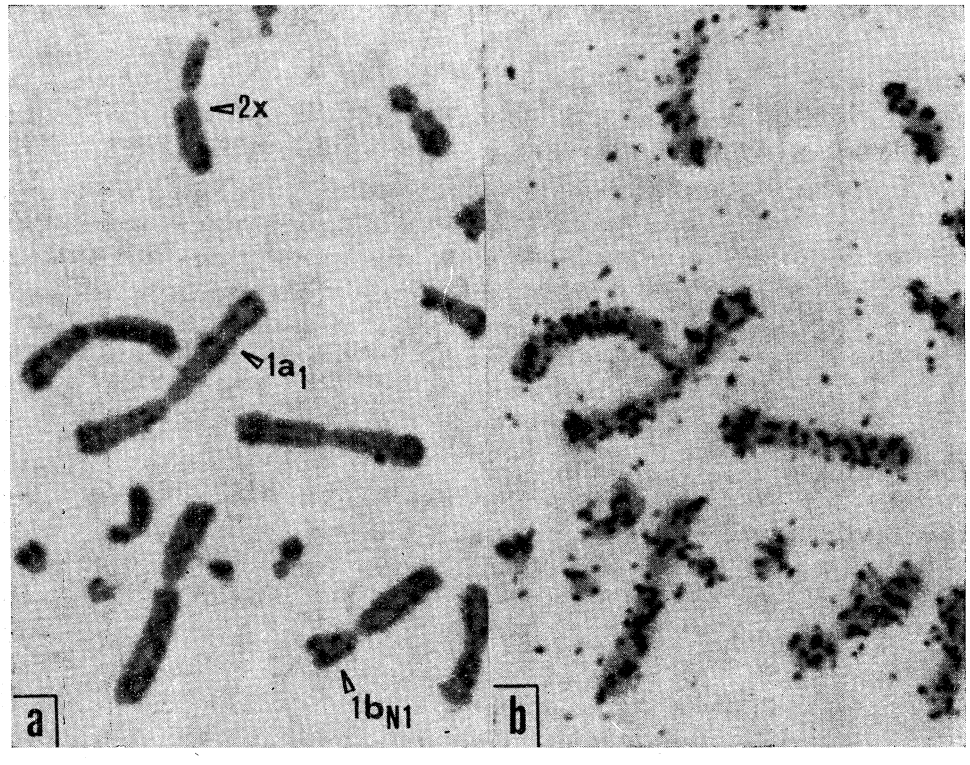

Fig. 1. – Partial metaphase of Chinese hamster cell strain C–125 treated with ^{3}H-actinomycin-D, *a*) after and *b*) before film removing.

us to distinguish the upper arm from the lower with absolute certainty. We therefore gave particular attention to chromosomes $1a_1$ and $2x$ which belong to the original karyotype of the species and chromosome $1b_{N1}$ which is a line marker [13, 17].

Heavily labelled metaphases were obtained from preparations which were exposed for many days and moderately labelled metaphases were obtained from preparations exposed for 2 or 3 days. Labelled karyotypes and the unlabelled correspondents of both types of metaphase were constructed and the labelling distribution was thoroughly studied above all for the three chromosomes indicated above.

A study of grain distribution on the chromosomes of the more heavily labelled cells did not reveal any constant labelling pattern, and thorough analysis of the chromosomes of 20 cells with labelling of medium intensity (fig. 1) made it possible for us to identify, along the three chromosomes indicated, areas of constantly heavier or lighter labelling and areas only occasionally labelled, but not areas constantly devoid of labelling.

The labelling patterns of chromosomes $1a_1$, $1b_{N1}$ and $2x$ can be seen in fig. 2. The same figure shows the despiralization patterns of the same chromosomes obtained with H. 33258 when the compound has been supplied to cells in culture.

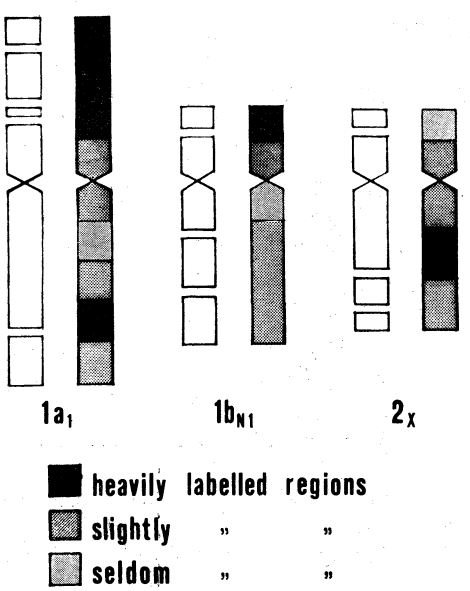

Fig. 2. – Idiogram of the three analysed chromosomes $1a_1$, $1b_{N1}$ and $2x$ showing for each chromosome: (left) the decondensation pattern for H. 33258 treatment and (right) labelling pattern for ^{3}H-actinomycin-D.

A comparison of the two patterns has showed that areas of H. 33258 induced decondensation do not correspond to areas lightly or occasionally labelled, but, except for the distal part of the short arm of the $2x$ chromosome, the despiralization regions correspond to areas which are always labelled.

The results can be explained with the data of Comings and Mattoccia (1972) who were not able to detect satellite fractions particularly rich in AT

bases in Chinese hamster DNA. A good number of GC bases would therefore be present along all the Chinese hamster chromosomes. Nor are these results in contrast with the data of Evenson *et al.* (1972) who have demonstrated the existence of very short segments of AT-rich DNA's in this organism, as these segments could correspond to the AT-rich areas despiralized by H. 33258 and be so short as to not be detectable when not despiralized at the chromosomal level, with the autorodiagraphic method.

It is necessary, however, to remember that the AMD bond with DNA is mediated by the presence of nuclear proteins. It has in fact been demonstrated that their total or partial removal by sulphuric acid or acetic acid [2, 23, 21, 20] or by proteolytic enzymes [10] increases the ^{3}H-AMD uptake.

In our study the hamster chromosomes underwent treatment with acetic acid during fixing (methanol: acetic acid, 3 : 1) According to Comings and Avelino (1974) and Brody (1974) this treatment should have removed only part of the hystones and of the non-hystone nuclear proteins. Therefore it cannot be excluded that the remaining proteins in some way affected the ^{3}H-AMD labelling pattern, preventing an exact evaluation of the distribution of the bases along the chromosomes.

References

[1] S. Avanzi (1972) – *Pattern of binding of tritiated actinomycin*-D *to onion chromosomes in fixed material*, « Rend. Acc. Naz. Lincei », *52*, 215–219.

[2] L. Berlowitz, D. Pallotta and C. H. Sibley (1969) – *Chromatin and histones: binding of tritiated actinomycin*-D *to heterochromatin in mealy bugs*, « Science », *164*, 1527–1529.

[3] N. Bianchi, A. Lima-de-Faria and H. Jaworska (1964) – *A technique for removing silver grains and gelatin from tritium autoradiography of human chromosomes*, « Hereditas », *51*, 207–211.

[4] Brody Th. (1974) – *Histones in cytological preparations*, « Exptl. Cell Res. », *85*, 255–263.

[5] P. G. Cionini (1973) – *Differential binding of* ^{3}H-*actinomycin*-D *as compared to other banding patterns in* Vicia faba *metaphase chromosomes*, « Caryologia », *26*, 541–547.

[6] P. G. Cionini and S. Avanzi (1972) – *Pattern of binding of tritiated actinomycin*-D *to* Phaseolus coccineus *polytene chromosomes. I. Nucleulus organizing chromosomes.* « Exptl. Cell Res. », *75*, 154–158.

[7] D. E. Comings (1975) – *Mechanisms of chromosome banding.* VIII. *Hoechst 33258*–DNA *interaction*, « Chromosoma (Berl.) », *52*, 229–243.

[8] D. E. Comings and E. Avelino (1974) – *Mechanisms of chromosome banding*, « Exptl. Cell Res. », *86*, 202–206.

[9] D. E. Comings and E. Mattoccia (1972) – DNA *of mammalian and avian heterochromatin*, « Exptl. Cell Res. », *71*, 113–131.

[10] L. Desai and R. Tencer (1968) – *Effects of histones and polylysine on the synthetic activity of the giant chromosomes of salivary glands of dipteran larvae*, « Exptl. Cell Res. », *52*, 185–197.

[11] D. P. Evenson, W. A. Mego and J. H. Taylor (1972) – *Subunits of chromosomal* DNA. I. *Electron microscopic analysis of partially denatured* DNA, « Chromosoma (Berl.) », *39*, 225–235.

[12] J. HILWIG and A. GROPP (1973) – *Decondensation of constitutive heterochromatin in L. cell chromosomes by a benzimidazole compound* (« *33258 Hoechst* »), « Exptl. Cell Res. », *81*, 474–477.

[13] H. KATO and T. H. YOSIDA (1972) – *Banding pattern of Chinese hamster chromosomes revealed by new technique*, « Chromosoma (Berl.) », *36*, 272–280.

[14] C. P. MILES (1970) – *Labelling and other effects of actinomycin-D on human chromosomes*, « Proc. Nat. Acad. Sci. (Wash.) », *65*, 585–592.

[15] W. MULLER and D. M. CROTHERS (1968) – *Studies of the binding of actinomycin and related compounds to* DNA, « J. Mol. Biol. », *35*, 251–290.

[16] G. OLIVIERI, A. ROCCHI, G. MATARESE and F. PALITTI (1971) – *Chromosome studies on polyploid cell strains of Chinese hamster*. I: *4x cell strains*, « Caryologia », *24*, 85–97.

[17] F. PALITTI, M. RIZZONI, P. PERTICONE, R. DE SALVIA and G. OLIVIERI (1974) – *Chromosome studies on polyploid cell strains of Chinese hamster*. V: *banding pattern*, « Caryologia », *27*, 237–253.

[18] S. PIMPINELLI, M. GATTI and A. DE MARCO (1975) – *Evidence for heterogeneity in the heterochromatin of* Drosophila melanogaster, « Nature », *256*, 335–337.

[19] E. REICH and J. H. GOLDBERG (1964) – *Actinomycin and nucleic acid function*, « Prog. Nucl. Acid. Res. Mol. Biol. », *3*, 183–234.

[20] A. ROCCHI BRASIELLO and M. DI CASTRO (1974) – *Actinomycin*-D *binding in spermatogenesis cells of* Asellus aquaticus, « Caryologia », *27*, 339–347.

[21] A. ROCCHI, F. GIGLIANI, A. DE CAPOA and N. ARCHIDIACONO (1974) – *Labelling of human chromosomes with* ^{3}H-AMD, « Humangenetik », *24*, 297–301.

[22] A. ROCCHI, G. PRANTERA, S. PIMPINELLI and M. DI CASTRO – *Effect of Hoechst 33258 on Chinese hamster chromosomes*. « Chromosoma (Berl.) », in press.

[23] M. SIEGER, G. GARWEG and H. G. SCHWARZACHER (1971) – *Constitutive heterochromatin in* Microtus agrestis: *Binding of actinomycin*-D *and transcriptional inactivity*. « Chromosoma (Berl.) », *35*, 84–98.

[24] H. M. SOBELL (1974) – *How actinomycin binds to* DNA, « Scient. Am. », *231*, 82–91.

[25] B. WEISBLUM and E. HAENSSLER (1974) – *Fluorometric properties of the bibenzimidazole derivative Hoechst 35258, a fluorescent probe specific for* AT *concentration in chromosomal* DNA, « Chromosoma (Berl.) », *46*, 255–260.