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Pattern of binding of tritiated actinomycin D to onion chromosomes in fixed material

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SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

Citologia. — *Pattern of binding of tritiated actinomycin D to onion chromosomes in fixed material* (*). Nota di SILVANA AVANZI, presentata (**) dal Corrisp. F. D'AMATO.

RIASSUNTO. — Apici radicali di *Allium cepa*, fissati e preparati secondo la tecnica dello striscio, sono stati trattati con actinomicina D tritiata. È stata eseguita una analisi autoradiografica per determinare quali zone cromosomiche, in cellule arrestate in metafase, leghino l'antibiotico. L'indagine ha messo in evidenza che ogni cromosoma di *Allium cepa* presenta in metafase un modello caratteristico di marcatura; modello evidente principalmente dopo un solo giorno di esposizione alla emulsione fotografica. I risultati ottenuti vengono discussi alla luce della specificità del legame della actinomicina col DNA e delle possibili condizioni limitanti la reattività della cromatina con l'antibiotico. Le osservazioni effettuate dimostrano che la marcatura da actinomicina in cromosomi metafasici può rappresentare un parametro molto utile in una analisi di cariotipo.

INTRODUCTION

Several autoradiographic studies have been carried out on the intracellular distribution of tritiated actinomycin D. Some of them have shown that it localizes in the nucleus [8, 13, 15]. The binding is limited to DNA as the treatment with DNase results in the total absence of label [5, 9]. The specificity of actinomycin D binding to DNA and its availability in tritiated form with high specific activity indicated it as a very sensitive means of detecting DNA, by autoradiography, in fixed cytological preparations. This technique has been applied to the detection of extra chromosomal DNA and its distribution even when it is unstainable by the Feulgen reaction [9, 10].

It is also known that actinomycin D binds selectively to guanine [6, 16, 17, 20, 21]. Its binding to DNA can, therefore, give indications on the distribution of guanine-rich DNA. Some authors, in fact, observed that labelling by actinomycin D is not homogeneously distributed in the nucleus [1, 9, 12, 26]. Thus, in the oocyte nucleus of *Xenopus laevis*, the cap region is the most intensely labelled [12] and in the polytene chromosome cells of the suspensor of *Phaseolus coccineus* the nucleolus organizing regions are

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(**) Nella seduta del 12 febbraio 1972.

the first to appear labelled by actinomycin D after short exposure to the photographic emulsion and are always more labelled than most of the genome at longer exposures [1]. These findings corroborated the idea that the more intensely labelled regions correspond to chromosomal loci with a particularly high guanine content.

The aim of the present work was to identify, by ^{3}H–actinomycin D, the pattern of distribution of G–C rich DNA along the metaphase chromosomes of *Allium cepa*.

Materials and methods

Roots from bulbs of *Allium cepa*, cv. Fiorentina, grown in tap water at room temperature were treated with a 0.3 % colchicine solution for 2 hours and fixed in alcohol–acetic acid 3: 1. The root tips were digested with a 5 % pectinase solution for 1 hour at 40° C and squashed on a slide. After removal of the coverslip by the dry-ice method, squashes were covered in the dark with a drop of a 5 mCi/l tritiated actinomycin D (AMD) solution (Schwarz Bioresearch, Orangeburg, U.S.A.), specific activity 8.4 Ci/mM, for 1 hour, rinsed in cold AMD (30 mgr/l) for 30 minutes and washed in water for 12 hours. The slides were then covered with Ilford L_4 emulsion and, after 1 to 5 days of exposure, developed, stained by methyl green-pyronine and mounted in Canada balsam.

Results and discussion

Plate I shows the labelling pattern by AMD of the eight chromosome pairs of *Allium cepa* after one day, three days and five days of exposure to photographic emulsion. The chromosomes are numbered according to Battaglia [2], to whom reference is made for a detailed description of the *Allium cepa* karyotype. In fig. 1, the observed labelling distribution has been drawn on the idiogramme. From both Plate I and fig. 1, it is seen that after one day only some chromosome regions are labelled, while after three days only a few regions are still unlabelled. After five days, almost all of the genome is labelled. Slight individual variations in labelling behaviour, in a few chromosome regions, have been detected.

Some literature data suggest that capacity of AMD binding to DNA is influenced by the state of repression of chromatin [3, 4, 22, 23]. Aside from the fact that the results on this relation are so far not univocal [25], this factor can be neglected in the condition of our study, because it is known that whole genome in the metaphase stage is devoid of transcription activity [7]. Therefore, binding of AMD to metaphase chromosomes can be evaluated in terms of AMD specificity for double-stranded helical DNA which contains guanine [6, 20, 21]. Consequently, chemically reactive loci may be located on metaphase chromosomes by AMD and identification of

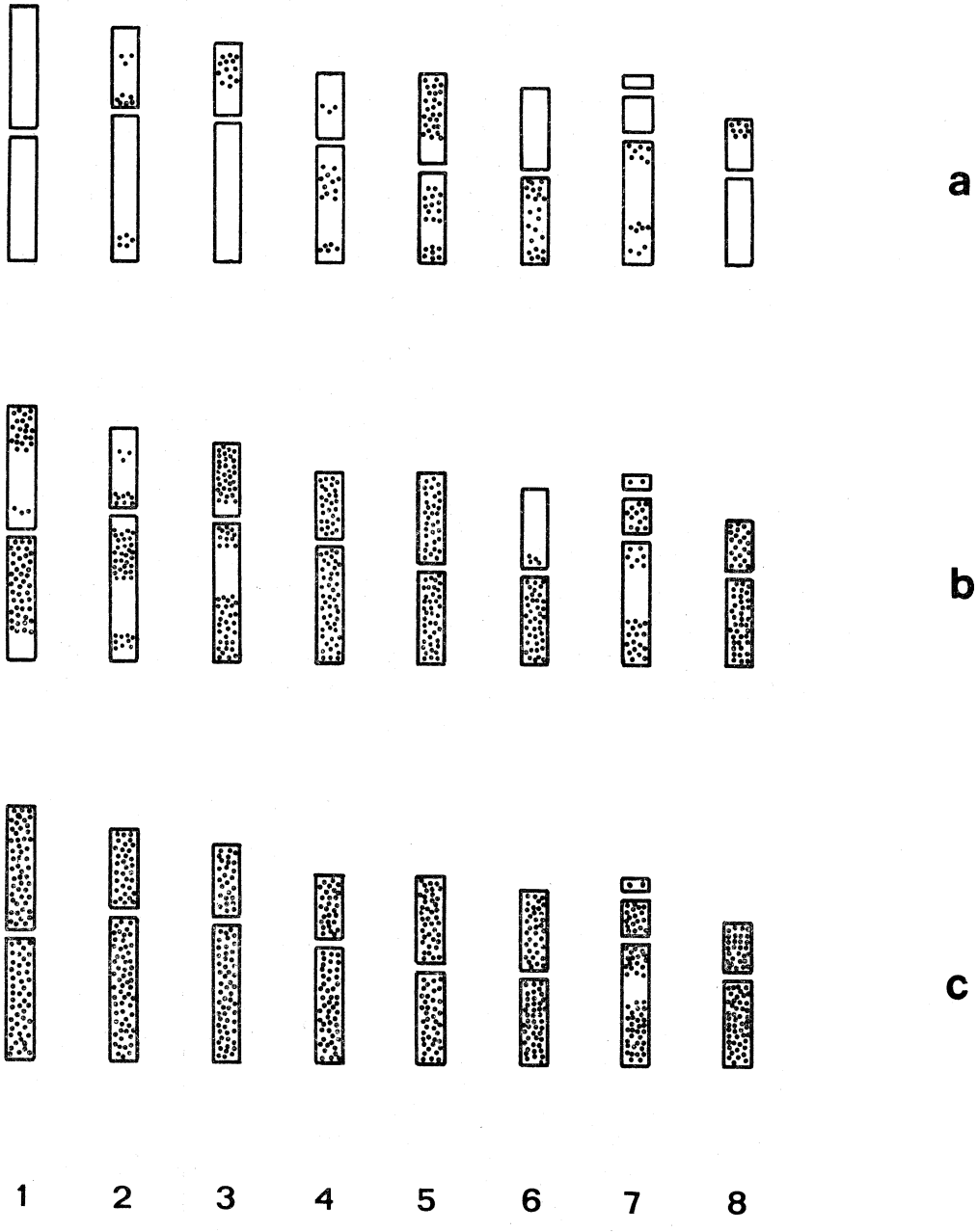

Fig. 1. – Haploid idiogram of *Allium cepa* showing the pattern of chromosomal labelling after one (a), three (b) and five (c) days of exposure following ^{3}H–actinomycin D binding. Magnification 2000× (Based on Battaglia 1957).

different chromosomes of a genome can be accomplished by the AMD labelling pattern. The present experiment, therefore, seems to indicate that the labelling detectable at the shortest exposure time makes manifest guanine-rich segments of DNA, while lack of labelling at the longest exposure delineates thymine-rich segments. In this connection, it is to be observed that the nucleolus organizing region of chromosome 7 of *Allium cepa* (fig. 1) shows no labelling after one day of exposure. Since it is known that ribosomal cistrons are localized in this chromosome region [11, 14, 24], it seems possible that a satellite heavier than rDNA is present in *Allium cepa*. Decision on this aspect is deferred until after the analysis of *Allium cepa* DNA, presently in progress. Other possibilities have nevertheless to be examined. A non random labelling pattern by AMD has been also detected by Miles in human chromosomes [19]. Besides the hypothesis that ribosomal cistrons be responsible for the binding of AMD to DNA, Miles also assumed that the distribution of different amounts and / or of different kinds of proteins along chromosomes may influence the reactivity of the DNA molecule to AMD. This supposition is supported by Kleiman and Huang's data [18]. These authors, in fact, observed that removal of proteins, mostly acid insoluble ones, results in a clear increase in binding of actinomycin D to DNA. The possibility cannot therefore be excluded that some guanine-rich sequences of DNA are prevented by proteins from their binding to actinomycin D.

As to the use of actinomycin D in karyotype analysis, the present observations show that the AMD labelling pattern at different exposure times may offer a useful means of distinguishing morphologically similar chromosome pairs at metaphase.

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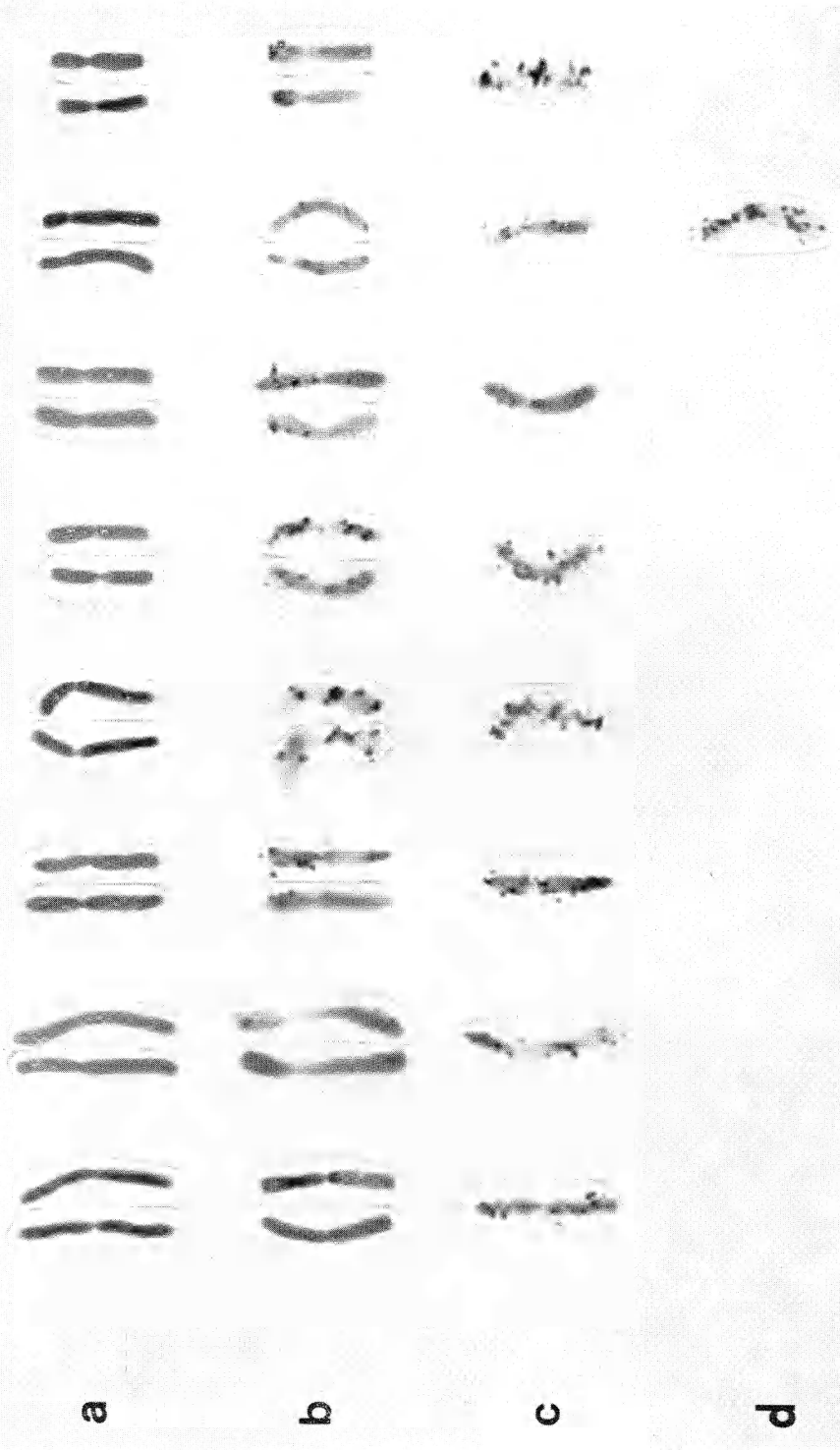

Allium cepa karyotype. Unlabelled chromosomes (a) and after one (b), three (c) and five (d) days of exposure following ^{3}H-actinomycin D binding; *d* shows the only chromosome having a still unlabelled region. Magnification 1500×.

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