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A study of the Ag-staining significance in mitotic NOR's

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The biological significance of nucleoli and mitotic Ag-staining NOR's has been the cause of controversy for several years. Whereas several authors state or assume a direct correlation between transcriptional activity and Ag-staining, others deny this hypothesis or claim a correlation between Ag-staining and decondensation of the NOR chromatin. Our results show that a decondensed state of the NOR chromatin is necessary but not sufficient to permit silver impregnation, allowing us to conclude that transcriptional NOR activity in the previous interphase is additionally required for silver staining.

INTRODUCTION

Silver nitrate is used by cytogeneticists for nucleolus and nucleolar organising region (NOR) staining, as a successful method of location. However, controversy about the biological significance of the silver staining has been raised over the last several years.

A direct correlation between the silver stainability and an active transcriptional state of the ribosomal genes has been generally admitted, and supported by many investigations (see Hubbell, 1985). Several authors however refute this interpretation, based either on biochemical studies of rRNA synthesis inhibition (Raman and Sperling, 1981; Goessens and Lepoint, 1982; Medina *et al.*, 1983; Sànchez-Pina *et al.*, 1984), or in the demonstration that there is no rRNA synthesis in metaphasic NOR's which, despite this, maintain their silver stainability (Hsu *et al.*, 1965; Fan and Penman, 1971; Goessens and Lepoint, 1979; Fakan and Puvion, 1980; Mirre and Sthal, 1981; Risueno *et al.*, 1982).

On the other hand, according to Sànchez-Pina *et al.* (1984), the inhibition of transcription has no effect on the condensation of the nucleolar chromatin; hence, Ag-staining reveals the existence of decondensed NOR chromatin which is transcriptionally inactive although capable of activation. Similar conclusions were reached by Medina *et al.* (1983) in plant cell investigations.

The present study describes the relation between the Ag-staining of NOR's and their decondensed state, by means of sequential observation of both Ag-stained NOR's and secondary constrictions.

MATERIALS AND METHODS

For this study, we have used bone marrow preparations from two species of mammals: *Talpa occidentalis* (Insectivora) and *Eliomys quercinus* (Rodentia). Both species show favourable karyotypic characteristics for this study because they have interstitially located NOR's which allow the visualisation of secondary constrictions. In both cases, chromosome preparations were done following Lee and Elder's (1980) methods.

For sequential staining we used the following method: (a) chromosome preparations were Ag-stained for 4–6 min. at 60°C in a moist chamber, with 100 per cent w/v silver nitrate solution, and mounted in 1:1 distilled water-glycerol. Slides were observed and photographed and then the cover-glass was removed by washing the preparations in running distilled water; (b) to remove the Ag-staining the slides were immersed in photographic intensifier (0·4 per cent potassium dichromate, 10 per cent HCl and distilled water, 10:10:5) and shaken until the complete loss of yellow coloration, and then rapidly immersed in conventional

photographic fixative for 10 minutes; (c) after washing, the preparations were stained for 5 minutes in 10 per cent Giemsa, $pH = 6{\cdot}8$. The same mitotic metaphase plates were then observed and photographed again.

RESULTS

The species *Talpa occidentalis* is a mole with a $2n = 34$ chromosome complement, with NOR's located in chromosome pair 3 (Jimènez *et al.*, 1984). A total of 40 cells of this species were analysed, representing a total of 80 NOR-bearing chromosomes. The results obtained in this study are summarised in table 1 and were subjected to a correlation analysis to investigate the possible interrelationship between both parameters studied (Presence of NOR-staining and secondary constriction), yielding a significant positive correlation ($r = 0{\cdot}698$, $t = 8{\cdot}61$, $p < 0{\cdot}01$).

Table 1 Chromosome account in relation to the presence (+) or absence (−) of both Ag-stained NOR's and secondary constrictions, in the species *Talpa occidentalis*

		Ag-stained NOR's +	Ag-stained NOR's −	
secondary constrictions	+	45	13	58
	−	0	22	22
		45	35	80

Eliomys quercinus is a rodent with $2n = 48$ chromosomes. NOR's are located in chromosome pairs 14 and 22. Sixty cells from this species were analysed with a total of 240 NOR-bearing chromosomes investigated. Table 2 summarises these results. As in *T. occidentalis*, statistical analysis showed a significant positive correlation between the presence of both Ag-stained NOR's and secondary constrictions ($r = 0{\cdot}693$, $t = 14{\cdot}83$, $p < 0{\cdot}01$).

In both species, as shown in figs 1(a) and (b), there was usually correlation not only between presence or absence of Ag-staining and secondary constrictions, but also between the size of these latter. In addition, there were some chromosomes which, although possessing secondary constrictions, do not show Ag-staining (figs. 1(c) and (d); table 1 and 2).

Table 2 Chromosome account in *Eliomys quercinus*. Notation as in table 1

		Ag-stained NOR's +	Ag-stained NOR's −	
secondary constrictions	+	171	30	201
	−	0	39	39
		171	69	240

DISCUSSION

Obviously, the significantly positive correlation between NOR stainability and the presence of a secondary constriction clearly supports the idea that the decondensed state of the NOR chromatin is a necessary prerequisite to its impregnation by silver (Medina *et al.*, 1983). Accordingly, in *T. occidentalis* as well as in *E. quercinus* chromosomes, there were no cases in which condensed secondary constrictions were Ag-stained. However, this decondensed condition, although necessary, seems to be insufficient, as there were 13 chromosomes of *T. occidentalis* and 30 of *E. quercinus* in which, in the presence of a visible secondary constriction (decondensed state), no Ag-staining was seen. On the other hand, there are several cases in amphibians in which secondary constrictions of variable appearance are frequently found which cannot be stained by silver (King, 1980). As King noted, these variable constrictions are presumably analogous to the cold-induced despiralised constrictions of Rudak and Callan (1976). Hence for positive Ag staining of NOR's, something more must be needed.

There are many investigations in which evidence for a direct correlation between NOR activity and Ag-staining has been found (see Hubbell, 1985).

On the other hand, although mitotic NOR's are known to be transcriptionally inactive and the inhibition of rRNA synthesis by cycloheximide (CHM) does not abolish silver staining (Medina *et al.*, 1983), it could be considered that transcriptional activity is certainly necessary for the Ag-stainability, but this need not occur precisely at the same time as mitotic chromosome fixation. Following Hubbell (1985), this staining may reflect silver stainable material trapped during chromosome condensation or residual rRNA gene activity below the level of detectability of the biochemical techniques used.

In conclusion, we suggest that for Ag-staining of mitotic NOR's, there are at least two prerequisites:

(a) NOR chromatin must be in a decondensed state and

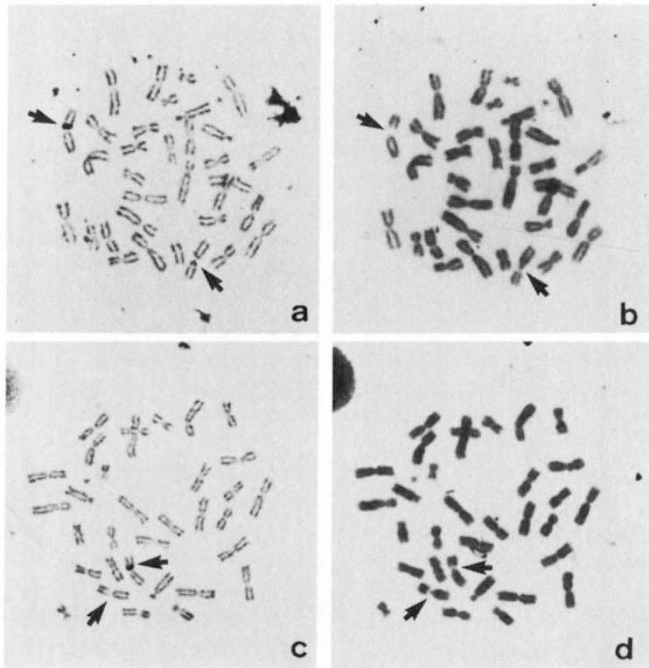

Figure 1 Sequentially stained cell from *T. occidentalis*. Ag-staining in a and c, and Giemsa staining in b and d. In the cell ab, NOR's are present on despiralised secondary constrictions. Staining is also correlated to size of constrictions. In the cell cd a chromosome is present which, having clearly despiralized its secondary constriction, has not however been ag-stained.

(b) this NOR must be transcriptionally active in the preceding interphase of the cell cycle.

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