The effect of RNA synthesis inhibitors on prenucleolar bodies[1]

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Summary. Inhibition of RNA synthesis prevents the fusion of prenucleolar bodies in the NOR region during nucleologenesis. However, their physical coalescence between themselves is increased, as shown in *Allium cepa* L. meristems.

At the start of a new cycle, the reforming nucleolus incorporates ribonucleoprotein material from the premitotic nucleolus[2]. This material apparently makes up the prenucleolar bodies which are scattered in telophase nuclei. Their appearance seems to precede any detectable transcription of NORs[3] and is not affected either by the inhibition of postmitotic RNA synthesis[4], or by the absence of NOR[5-7]. Experimentally, if we prevent reinitiation of RNA synthesis in telophase[4,8,9] posttelophase nuclei will remain with scattered prenucleolar bodies forever. However, there appears to be some fusion between prenucleolar bodies themselves which is never observed in control nucleologenesis[4]. This fusion of prenucleolar bodies may occur either spontaneously due to the increased time they stay in the nuclei or by a direct effect of the RNA synthesis inhibitors on the prenucleolar bodies' adhesiveness. In this paper we have tried to decide between these 2 possibilities, by comparing the behaviour of prenucleolar bodies in anucleolate and tetraploid nuclei produced by colchicine treatment as well as in synchronous binucleate cells produced by caffeine, both in the presence and absence of RNA synthesis inhibitors.

Material and methods. The material used was meristematic cells of *Allium cepa* L. roots grown at 10 ± 0.5 °C in tap-

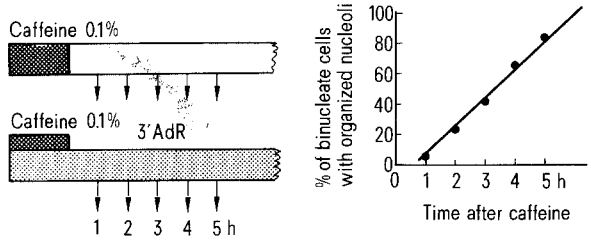

Fig. 1. Experimental schemes used to analyse nucleologenesis in control binucleate cells (upper row), and in 3'AdR (or EB) treated binucleate cells (lower row). Nucleologenesis kinetics in control binucleate cells (right).

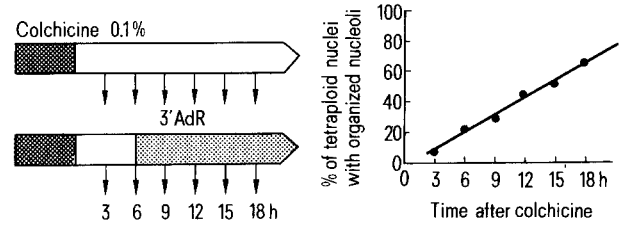

Fig. 2. Experimental schemes used to compare nucleologenesis in tetraploid cells produced by colchicine (upper row) and the effect of 3'AdR in a similar experiment. Nucleologenesis kinetics in tetraploid nuclei (right).

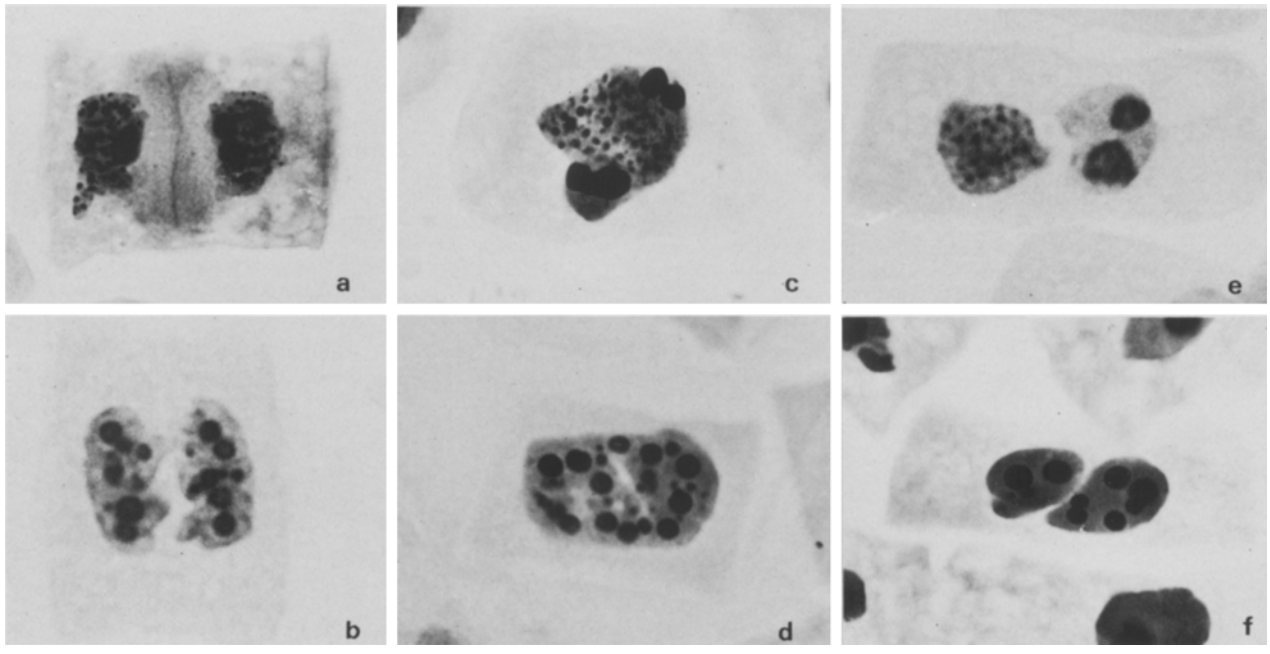

Fig. 3. *a* Control telophase in reorganization with the incipient nucleoli and a large number of small prenucleolar bodies. *b* Binucleate cell treated with 3'AdR (as shown in the lower scheme of figure 1) showing enlarged prenucleolar bodies 6 h after the end of caffeine labelling. *c* Tetraploid nucleus in reorganization showing the 4 incipient nucleoli and a large number of small prenucleolar bodies. *d* Tetraploid nucleus produced by colchicine in which nucleologenesis is inhibited by 3'AdR treatment as shown in the lower row of the figure; enlarged prenucleolar bodies can be seen. *e* Control aneuploid nuclei one of them with fully organized nucleoli and the smallest one showing only non-fused prenucleolar bodies 24 h after colchicine treatment. *f* Aneuploid nuclei, treated with 3'AdR as shown in lower row of figure 2, showing fused prenucleolar bodies 24 h after colchicine.

water which was renewed every 24 h and aerated continuously by bubbling air (10–20 ml/min). The roots, still attached to the bulbs, were immersed in the different treatment solutions. 1 h treatment with 0.1% colchicine was used to produce tetraploid and aneuploid nuclei. 0.1% caffeine treatment for 2 h produced a binucleate population which entered interphase synchronously[10]. 10^{-4} M 3'-deoxyadenosine (3'AdR) or 100 µg/ml ethidium bromide (EB) were used to inhibit RNA synthesis. The roots were fixed in 10% formol:1% hydroquinone (1:1) for 1–2 h at room temperature, and the silver impregnation technique[11] was used for the staining of nucleoli and prenucleolar bodies.

Results and discussion. *Allium cepa* L. has only 1 pair of NOR which form 2 nucleoli. Nucleologenesis kinetics in control binucleate cells (figure 1) is obtained by scoring the frequency of binucleate cells with fully organized nucleoli, and then without any prenucleolar bodies, at different times after the end of caffeine labelling. Figure 3,a, shows a control cell in the process of reorganization, with the 2 incipient nucleoli and a large number of small prenucleolar bodies which appear to cover the chromosomes. When postmitotic RNA synthesis is inhibited by continuous treatment with 3'AdR or EB (as shown in figure 1) the nucleolus fails to form. The posttelophase nuclei can be seen to be full of prenucleolar bodies (figure 3,b) which are fewer but larger than prenucleolar bodies in control cells. Segregation of the prenucleolar bodies as well as the appearance of internal vacuoles are seen in some cells. Colchicine treatment induces tetraploid and multinucleated meristematic cells (figure 2). Cells with only 1 single tetraploid nucleus have extremely delayed nucleologenesis kinetics, since only 50% of the tetraploid mononucleate cells have fully organized nucleoli 12 h after the end of the colchicine treatment (see figure 2). Nevertheless, as seen in figure 3,c, these cells have small prenucleolar bodies that are non-fused even after 8 h of being in the nuclei. Moreover, in multinucleated tetraploid cells in which one of the nuclei does not contain any nucleolar organizer, chromosomes still have small non-fused prenucleolar bodies 24 h after colchicine as seen in figure 3,e. Hence the time prenucleolar bodies remain scattered in a cell does not modify their fusion properties. Tetraploid cells also treated with colchicine but submerged in a 3'AdR solution from the 6th h of the recovery (figure 2), had enlarged prenucleolar bodies (figure 3,d) when compared with cells not treated with the inhibitor after the same number of h. Figure 3,f, shows a multinucleate tetraploid cell 24 h after the end of colchicine treatment, where prenucleolar fusion is also evident when compared with control conditions (figure 3,e).

These data suggest that treatment with RNA synthesis inhibitors produces adhesiveness in prenucleolar bodies between themselves. This functional characteristic may be related to the finding that these prenucleolar bodies show ultrastructural features which make them different from those found in untreated meristems. Hence, the prenucleolar bodies of 3'AdR-treated cells show a cap formed by granules, resembling a sort of segregation where the granular component is scarce[12]. These changes in fusion properties of the prenucleolar bodies pose an intriguing question about the role of newly synthesized RNA in their assembly properties.

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2 A. Lepoint and G. Goessens, Exp. Cell Res. *117*, 89 (1978).
3 G. Morcillo, C. de la Torre and G. Giménez-Martín, Exp. Cell Res. *102*, 311 (1976).
4 G. Morcillo and C. de la Torre, Biol. cell. *36*, 1 (1979).
5 D.D. Brown and J.B. Gurdon, Proc. natl Acad. Sci. USA *70*, 1955 (1973).
6 S.G. Philips and D.M. Philips, Exp. Cell Res. *120*, 295 (1979).
7 D. Hernández-Verdún and M. Bouteille, VI Eur. Nucleolar Workshop Weimar, 1979, p.28.
8 G. Giménez-Martín, C. de la Torre, M.E. Fernández-Gómez and A. González-Fernández, J. Cell Biol. *60*, 502 (1974).
9 J.C. Stockert, M.C. Fernández-Gómez, G. Giménez-Martín and J.L. López-Sáez, Protoplasma *60*, 265 (1970).
10 G. Giménez-Martín, A. González-Fernández and J.F. López-Sáez, J. Cell Biol. *26*, 305 (1965).
11 M.E. Fernández-Gómez, J.C. Stockert, J.F. López-Sáez and G. Giménez-Martín, Stain Technol. *44*, 48 (1969).
12 A.M. Sacristán-Gárate and J.C. Stockert, Experientia *30*, 294 (1974).

Satellite DNA sequences and reproductive isolation in the *Drosophila willistoni* group[1]

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Summary. Satellite DNAs of the *Drosophila willistoni* group have been analyzed by Hae III endonuclease digestion. Differences were observed between the 2 species *D. willistoni* and *D. paulistorum* and also between semispecies of *D. paulistorum* and, to a lesser extent, between subspecies of *D. willistoni*. Differences did not appear to be consistently greater between strains producing sterile hybrid males than between those producing fertile hybrids.

Satellite DNAs are highly repeated sequences mainly located in heterochromatin but also in telomeric sites of eukaryotic chromosomes. The different hypotheses proposed about their function include 1. that they hinder recombination in the chromosome sections where they are located[2] and 2. that they play a role in speciation by hindering the pairing of homologous chromosomes during meiosis in interspecific hybrids[3–5]. The present work tests this 2nd hypothesis by studying highly-repeated satellite sequences in populations at different stages of evolutionary divergence.

The *Drosophila willistoni* group of species provides ideal material for the test, because the main stages of the speciation process can be identified within the group[6]. We have examined the satellite DNA sequences in 8 different strains: 1. *D. willistoni willistoni* from Santa Marta (Colombia); 2. *D. w. willistoni* from Tame (Colombia); 3. *D. willistoni quechua* from Lima (Peru); 4. *D. w. 'quechua'* from