great vagility generally have much more homogeneous karyotypes than those of reduced vagility because of the smaller probability of any chromosome mutation being fixed. On the other hand, the modal chromosome number of *Cryptocephalus*, 2n = 30, enables a clear separation with respect to other species of Cryptocephalinae, like those of *Pachybrachis*, with 2n = 16, or those of the close Clytrinae subfamily, with 2n = 22-24 mostly[14,15]. A common karyological trait of the 2 major groups of Camptosomata chrysomelids, Cryptocephalinae and Clytrinae, is the small size of their chromosomes, a characteristic which is also shared with those of Megalopodinae[16], the presumed ancestor subfamily of both groups.

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The nucleolar organizer in meristem cells of *Allium cepa* L. bulbs

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Summary. The nucleolar organizer (NOR) was studied in nucleoli of dormant meristems cells of *Allium cepa* L. bulbs by means of electron and light microscopy. Nucleoli are mainly fibrillar and they present a clear segregation between fibrillar and granular components. In these cells, the NOR, whose electron density is lower than the NOR heterochromatin, appears as a compact single mass located inside the nucleolus. These ultrastructural characteristics make NOR visualization possible at the light microscopic level by the complementary use of both silver impregnation and Feulgen staining techniques.

The structure of the nucleolar organizer (NOR) has been extensively studied at the ultrastructural level, both in animal and plant cells[2,3]. Basically, it is formed from a bundle of chromatin fibers penetrating into the nucleolar mass[4-6].

The nucleolar ultrastructure pattern varies according to the functional stage of the cell[7,8], appearing segregated in the dormant cells of *Allium cepa* L. seeds and bulbs[9-11]. It has been suggested that the nucleolar organizer itself participates in the mechanism of segregation[3,6,12]. However optical visualization of the NOR in interphase nuclei, in relationship with other nucleolar components, has not been extensively studied. The present report deals with the visualization of the NOR at light microscopy level in dormant meristem cells of *Allium cepa* L. bulbs. These optical images are compared with those observed at the ultrastructural level.

Material and methods. Root primodia were dissected from the crowns of *Allium cepa* L. bulbs. Silver impregnation, according to Fernández-Gómez et al.[13] and Feulgen staining, with hydrolysis in 5 N HCl at 20 °C for 1 h, were used for the NOR study at the light microscopic level. After staining, squashes were prepared from the terminal 2 mm of each root. Permanent mounting was carried out following Conger and Fairchild's method[14]. For electron microscopy, the 2nd mm from the root tips were fixed in 2% glutaraldehyde in 0.025 M cacodylate buffer pH 7.2 for 2 h and postfixed in 1% osmium tetroxide in the same buffer for 1 h. Afterwards, the root segments were embedded in Epon 812 according the current procedures. Ultrathin sections were stained with uranyl acetate and lead citrate. The preparations were examined with a Phillips EM-300 electron microscope.

Results. Figures 1 and 2 show ultrastructural views of the nucleolus in the dormant meristem cells. It can be seen that the nucleolus is very compact, and the segregation of the nucleolar components is plainly apparent where the fibrillar part is surrounded by a very thin rim of granules. Penetration of a less dense material into the nucleolar mass is clearly seen. The morphological pattern of this region coincides with the one previously described as the NOR in *Allium cepa* L.[6,11]. The NOR zones are continuous with the adjacent chromatin mass, called NOR-associated chromatin or NOR heterochromatin by Phillips[15]. In some cases, 2 of these masses are associated with a single intranucleolar NOR (fig. 2).

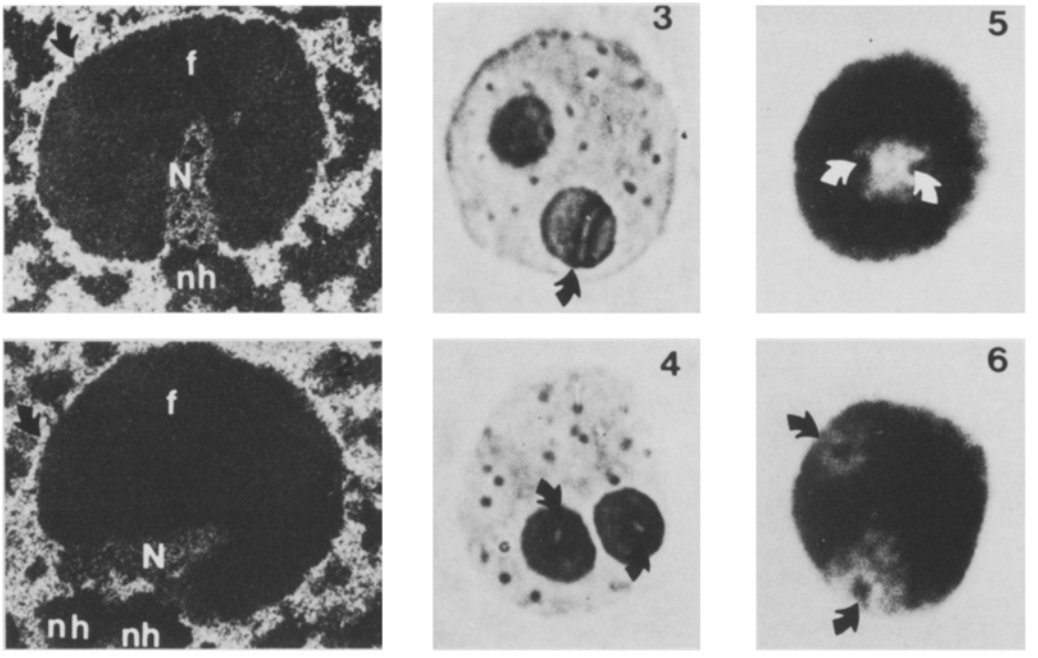
Figures 3 and 4 show meristem cell nuclei as they appear in dormant unsprouted roots after silver impregnation, which preferentially contrasts the fibrillar nucleolar component. A very compact nucleolus is penetrated by a clear zone corresponding to a less stained material. This material crosses the nucleolus either completely (fig. 3) or, more often, partially (fig. 4).

Figures 5 and 6 show nuclei from similar meristem cells as they appear after Feulgen staining. The nucleoli are unstained though Feulgen positive masses are detected within them. In some cases, a Feulgen-positive mass seems to penetrate the nucleolus (see arrows, fig. 5 and 6).

When 2 nucleoli are present, a Feulgen positive mass is present in each of them (fig. 6). However, in nuclei where both nucleoli have fused in a single nucleolus 2 such Feulgen-positive masses are detected (fig. 5).

The Feulgen-positive areas correspond to the NOR heterochromatin while intranucleolar NOR is not evident. On the other hand, silver impregnation allows us to follow the channel occupied by intranucleolar NOR which remains unstained.

Discussion. The nucleolar organizer (NOR) has been described in meiotic cells[15,16] and in somatic metaphase chromosomes by silver impregnation[17] and by the N-banding technique[18]. However these techniques do not provide information about the structural relationships between the



Figures 1 and 2 show ultrastructural views of the nucleolus in dormant cells; N, nucleolar organizer region (NOR); nh, NOR heterochromatin; f, fibrillar component of the nucleolus. The arrows point out a thin rim of granular component. × 13,000. Figures 3 and 4 show nuclei of dormant cells after silver impregnation. The nucleolus is positively stained. Arrows point to less-stained material inside the nucleolus. That probably corresponds to the nucleolar organizer region (NOR). × 2000. Figures 5 and 6 show nuclei of dormant cells after Feulgen staining. In these cases the nucleoli appear unstained. Arrows indicate the position of Feulgen-positive bodies within the band of the nucleolar zone. These bodies probably correspond to the NOR heterochromatin. × 2000.

NOR and the rest of the nucleolar components. In this report, it is shown that the NOR can be visualized at the light microscopic level by the complementary use of silver impregnation and Feulgen staining (figs 3–6).

As is known, silver impregnation preferentially stains some nucleolar protein which is concentrated in the fibrillar portion of the nucleolus[13], whereas the Feulgen reaction is specific for DNA. Considering these facts the Feulgen positive masses inside the nucleoli at light microscopy level should correspond to the NOR heterochromatin while the silver-clear area, also inside the nucleolus, should correspond to the channel where the NOR is inserted.

There are 3 factors in these dormant cells that make NOR visualization possible at the light microscopy level, namely: the degree of chromatin condensation, nucleolar segregation and the sparse occurrence of a granular component.

Chromatin is more condensed in dormant cells than in proliferating cells[19]. Cells with a high metabolic activity present the 3 nucleolar components intermingled[3,20,21]; in contrast, dormant cells, that have low activity, present the nucleolar components segregated with only a little of the granular component[9–11]. There seems to be a close relationship between the degree of chromatin condensation and nucleolar segregation. Chromatin condensation by some sort of coiling and supercoiling amplifies NOR compaction and retraction. The NOR condensation produces a displacement of the fibrillar component of the nucleoli to a more central position, thus originating the segregation of the nucleolar components[5,12]. This is a possible mechanism for the appearance of the clear channel-shaped zone detected after silver impregnation and the Feulgen-positive masses inside the nucleolus, corresponding to the NOR and NOR heterochromatin, respectively.

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