Nucleolar Structure in Root-Tip Cells of *Allium cepa*

Although silver impregnation of the nucleolus in animal cells had already revealed varying degrees of argentophilia in their components[1-3], the nucleolus of plant cells appeared to show rather homogeneous results from silver staining[4-6].

We have carried out a number of observations on the structure of the nucleolus in root-tip cells of *Allium cepa* by applying silver-staining technique for plant cells[7], as well as staining with basic fuchsin at 0.2% following fixation with 10% formol.

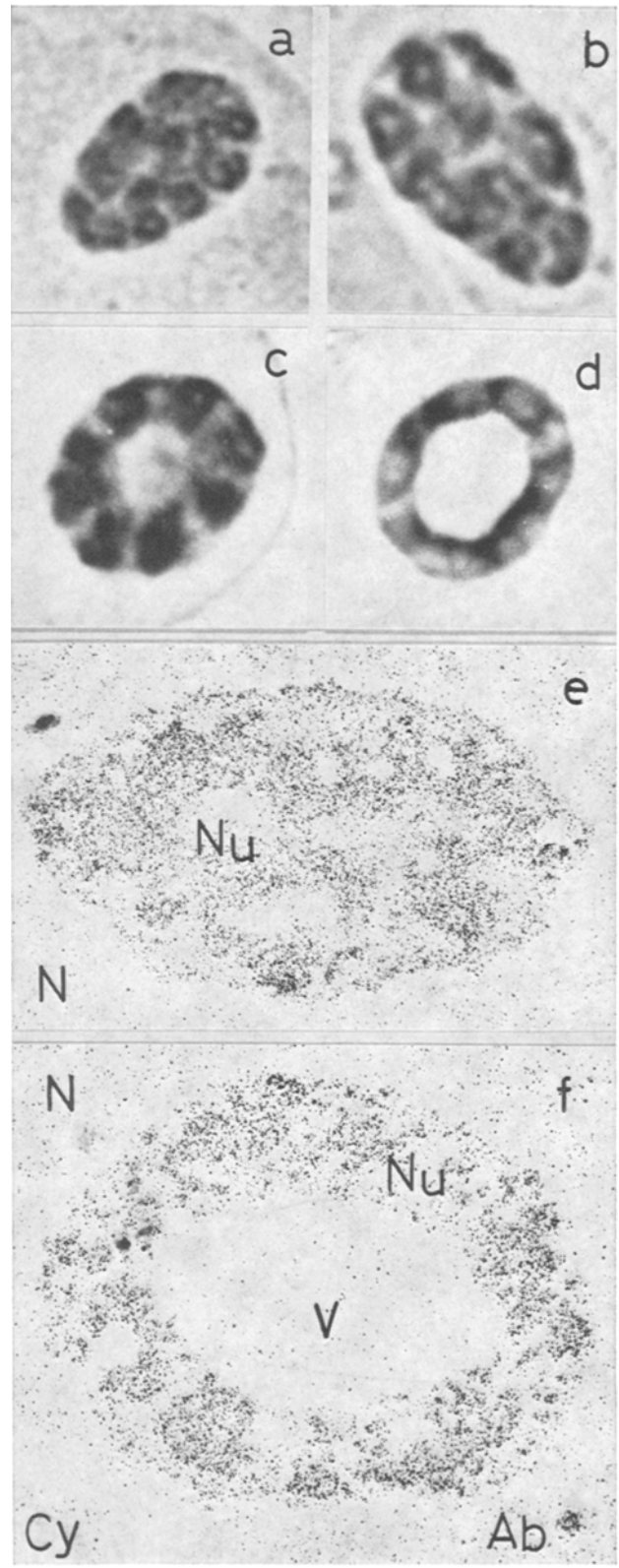
With the silver-staining technique, all the nucleoli seem to consist of 2 components: one highly argentophilic (dark brown) in the form of irregular-shaped granules about 0.5 μ in diameter, the other, less argentophilic (pale yellow) surrounding the dark brown granules, which are arranged in different ways according to the morphological characteristics of the nucleolus. In compact nucleoli, the argentophilic granules are fairly evenly distributed (Figure a and b), whereas in nucleoli which have a central vacuole they are situated on the periphery (Figure c). In both cases we occasionally observe areas of low argentophilia, in the interior of the granules.

Part of the silver-stained roots were dehydrated in an acetone series and embedded in Durcupan ACM (Fluka), to be studied under the electron microscope. To obtain the ultra-thin sections an Ultrotome LKB was employed. The observations were made with a Siemens Elmiskop I, and the pictures taken on Scientia Gevaert plates. The distribution of more and less argentophilic components was confirmed in the ultrathin sections by the presence of alternating areas of different density in the silver grains (Figure e and f). Occasionally there appeared to be continuity between the chromatin and the small vacuoles inside the areas which were rich in grains of silver. We also observed the presence of certain small highly impregnated bodies inside the nucleus.

With the basic fuchsin staining, the nucleoli appeared as the counterpart of those stained with silver (Figure d). The most basophilic areas are arranged in fairly continuous fashion, with less basophilic spaces inside them, corresponding morphologically to the silver-stained granules.

The argentophilic component of the nucleolus seems to be proteinic in character[4], and morphologically similar to the prenucleolar bodies to be found in the telophase. Our observations show that the argentophilic component does not necessarily take up a linear arrangement, as described in the case of the nucleolonema[3], but appears rather as a discontinuous structure, as described by other authors[8,9].

From the ultrastructural standpoint, the argentophilic component seems to be made up of the fibrillar parts of the nucleolus[10] which contain a concentrated matrix of



Nucleoli of root-tip cells of *Allium cepa*. (a), (b) and (c) Silver stain, bright-field lighting, × 3000. (d) Basic fuchsin stain, phase contrast, × 3000. (e) and (f) Silver stain, electron microscopy, × 17,000. N, nucleus; v, central vacuole; Cy, cytoplasm; Ab, argentophilic body; Nu, nucleolus.

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protein[11]. On the other hand, the more basophilic component of the nucleolus seems to correspond to the granular areas[12].

Two kinds of vacuole-like structure have been described in vegetable nucleoli. The central vacuole[13] is not a constant feature, but represents rather some functional character[14], while the small vacuoles described within the fibrillar areas[13] seem to correspond to the intranucleolar chromatin (see review by LAFONTAINE[15]).

Our observations show that the components of the nucleolus have different staining affinities in plant cells and suggest that the argentophilic and the basophilic components correspond, respectively, to the following structures observed in animal cells: argentophilic spherules and interstitial matter[1]; argentophilic granules and fundamental substance[2]; nucleolonema and pars amorpha[3].

Resumen. La tinción con plata y con fucsina básica del nucleolo en células meristemáticas de *Allium cepa* permite detectar la localización de dos componentes de distinta afinidad tintorial. El componente de mayor argentofilia aparece en forma de gránulos, distribuídos en el interior de una matriz contínua que presenta marcada basofilia.

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Ultrastructure of Human Myeloma Cells Studied by Peroxidase Conjugated Antibodies Directed to Human Immunoglobulin Component Chains

Multiple myeloma is a malignant disease characterized by neoplastic proliferation of cells of plasmacytic line. The clinical observation that a pathological protein can be demonstrated in the serum and urine of 90–95% of patients with plasma cell neoplasm is well known[1,2].

In general, morphology of myeloma cell is very similar to that encountered in non-pathological plasma cells. There are no pathognomonic cytological features which permit accurate differentiation of neoplastic from normal plasma cells, or of cells responsible for the production of the various pathological proteins[3–6].

In 1961, BESSIS[6] described paracrystalline structure with periodicity in apparently pathological plasma cells obtained from a patient with prolonged multiple myeloma. Although the authors suggested that these structures were formed of polymers of gamma globulin molecules produced in myeloma cells, actual nature of these crystalline structures and their functional significance were not elucidated.

Recently, a case of multiple myeloma was reported by Dr. S. ITO and his co-workers of the Anjo Hospital, Aichi (Japan), in which similar fine crystalline inclusions were demonstrated in the cytoplasma of plasmacytic cells of sternal bone marrow specimens and within kidney tubules of a 50-year-old female patient with advanced multiple myeloma.

In an attempt to elucidate the origin of these crystalline structures and their functional significance, we introduced in the present study a combination of electron microscopy and immunocytochemistry. Sternal bone marrow specimen was obtained by aspiration from this patient and treated with peroxidase-conjugated antibodies each monospecific for immunoglobulin heavy chain class (anti-α, anti-γ, anti-μ) or light chain type (anti-$\varkappa$, anti-λ).

Antibodies were specifically purified by exclusive use of solid immunoadsorbent conjugated with an appropriate antigen as described previously[7,8]. Conjugation of purified antibodies with horseradish peroxidase was performed by closely following the method of NAKANE and PIERCE[9]. Antibody-peroxidase complexes thus prepared were found to retain the reactivity of both antibody and peroxidase, because precipitin line produced between antigen and enzyme-labelled antibody in agar exhibited brownish colour when placed in enzyme substrate.

When glutaraldehyde-fixed cell sediment was treated with peroxidase-conjugated antibody directed to $\varkappa$-type light chain, the intracellular site of immunoglobulin accumulation can be cytochemically revealed under electron microscope as conspicuous and discrete electron dense precipitate[10,11]. The unresponsiveness of these myeloma cells to the peroxidase label of other antibody specificity agreed with the serological observations that the increase of only free $\varkappa$-type light chain level was detected in the serum and urine of the patient.

Immunoglobulin light chain of $\varkappa$-type was localized to ergastoplasm, its membrane, ribosomes lining the ergastoplasmic membrane and nuclear membrane (Figure

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