Ultrastructural Investigations of Nuclear Formations in Carnation Cells from Cultured Tissues

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Ultrastructural investigations on vegetal tissues obtained from different plants (Pteridophyta, Gymnospermae, Angiospermae) have demonstrated the presence of certain inclusions (Wergin et al. 1970) or paranucleolar corpuscular formations (Lafontaine 1965, Hyde 1967, Jordan and Chapman 1971, 1973, Jordan 1976). These were more frequently encountered in meristematic tissues. Nuclear formations were also observed in animal cells hormonally stimulated (Vagner-Capodano et al. 1978) or in those infected by viruses (Mihaiescu and Mișcalencu 1979, Recher et al. 1969, 1976).

Most authors have named these formations nuclear bodies. Sankaranarayanan and Hyde (1965) and later Hyde (1967) proposed the term karyosomes. Lafontaine (1965) named them spherical nuclear bodies, indicating that some of them have a loose structure similar to a thread ball. This structure has determined Jordan (1976) to apply the name of loose nuclear bodies to them as opposed to dense nuclear bodies, which have a similar structure to that of the nucleolus.

The nuclear bodies and inclusions were identified both in normal or experimentally stimulated cells and in cells taken from sick organisms. Their nature and role are not yet fully clarified. Some authors have demonstrated a viral nature in pathological cases (Mihaiescu and Mișcalencu 1979). Sjolund and Shih (1970) and Wergin et al. (1970) suppose that the nuclear inclusions should also have a viral origin. Other authors consider that the nuclear bodies are related to the functioning of the nucleolus (Recher et al. 1969, 1976), or as being its morphological expression during the periods of increased rRNA synthesis, following the action of certain stimulating factors (Jordan and Chapman 1971, 1973, Vagner-Capodano et al. 1978).

Nuclear formations in Dianthus caryophyllus L. have not been described until now. This paper will present our investigations regarding two types of nuclear formations observed in cell nuclei of cultured tissues as well as a discussion of their structure, origin and possible role.

Materials and methods

The vegetal material studied was taken from caulinar meristem callus of terminal buds of carnation (Dianthus caryophyllus var. Linda) aseptically cultured in vitro. To the Heller culture medium (macroelements, microelements, Fe, EDTA, 1 Communicated in the symposium of "Comparative Molecular and Cellular Biology", Bucharest, December 11–12, 1980.
glucose, vitamins B₁, B₆, PP, mesoinositol, agar-agar, 100 mg/l potassium phosphate and 1 mg/l 2, 4-D) the following substances were added: procaine (100, 10, 1 and 0.1 mg/l), β-indolyl acetic acid (0.250 and 0.100 mg/l) and β-naphthoxiacetic acid (1 and 0.1 mg/l).

After the induction of the formation of calluses (60 days from the initiation of cultures) these were transferred to flasks, on a sponge support in contact with a piece of filter paper through which the tissues were constantly fed another type of medium (Morel-Müller), to which 4 mg/l BAP (benzyl amino purine) and 2 mg/l NAA (naphthyl acetic acid) were added. Under these conditions, the calluses were grown for another 60 days. In the case of the media containing procaine, 2, 4-D, β-indolyl acetic acid or β-naphthoxiacetic acid, the initial cells of the meristem formed buds from which young plants developed having 1.5–2 cm in length and lacking the roots.

The vegetal material for electron microscopic examination was taken from both the callus zone and the plant leaflets. Fixing lasted for 2 hrs and was done in a solution of 3% glutaraldehyde adjusted to pH 7.2 by the use of 0.1 M phosphate buffer. Postfixing was done in 1% osmic acid. The samples, dehydrated in acetone solutions of increasing concentration, were embedded in vestopal W. The sections were cut with a LKB III ultramicrotome, contrasted with uranyl acetate and lead citrate and examined in a Tesla BS-613 electron microscope.

Results and discussions

The electron microscopic examination of the calluses and of the leaflets developed by them constantly showed the presence in the nucleus of an electrondense spherical formation, usually localized near the nucleolus (Figs. 1–3). Its diameter varies between 0.5 and 0.8 μm, i.e. close to the dimensions observed for such formations by Lafontaine (1965) in meristematic cells of Allium cepa, Vicia faba, Raphanus sativus and by Jordan (1976) in root phloem parenchyma of Daucus carota. Jordan (1976), citing several authors, mentions that similar formations were also described in Pisum sativum, Spirogyra sp., Plantago ovata, Zea mays, Crepis capillaris and Beta vulgaris. This formation is thus similar to the loose nuclear bodies described by the above cited authors (Lafontaine 1965, Jordan 1976).

The loose electrondense body appears sometimes either as separated from the nucleolus by a narrow band of karyoplasm (Fig. 2) or as tightly bound to the nucleolus (Fig. 3). However, the structure of the electrondense body is always different from that of the nucleolus. It appears as made of a mass of fibers which sometimes have a more or less parallel orientation (Fig. 2). On a favourable section, where several fibers have been cut transversally (Fig. 3, between the horizontal lines) it is obvious that these have an electrondenser central part (Fig. 3, large arrows), which measures 7 nm in diameter and a wall of 17 nm thickness. Hence, the transverse diameter of a fiber is 24 nm. At a larger magnification one can see that the fibers are made of fibrils whose diameter is approximately 16 Å (Fig. 3, little arrows). Both the fibers and the fibrils are not stuck together so that there is an amorphous material between them—a kind of fundamental matrix of the loose body. Usually, the
orientation of fibrils is very disordered.

The fact that Sankaranarayanan and Hyde (1965) have shown that these formations are made of granules of 30 nm is considered by us due to a description

Figs. 1-2. 1, loose electrondense body near the nucleolus. Carnation callus grown on a medium with procaine (10 mg/l). ×21,000. 2, nucleolus and a loose electrondense body with the section plane along the fibers; carnation leaflet of a callus grown on a medium with procaine (10 mg/l). ×38,000.
Figs. 3-4. 3, ultrastructure of a loose electrondense body in a transverse section. The fibers are 24 nm in diameter (between the horizontal lines) and the fibrils 1.6 nm (little arrows). Carnation callus treated with β-naphthoxiacetic acid (0.1 mg/l). ×176,000. 4, unelectrondense circular and oval areas in the cell nucleolus and nucleus from control meristematic callus. ×29,000.
obtained from a transverse section of the fibrils. We partly confirm the data reported by Jordan (1976) which considers that these loose nuclear bodies are constituted of fibers with a diameter of 30 nm and these, in turn, are composed of subfibers of 7 nm in diameter. Lafontaine (1965) reported that these bodies are made up of fibrils of 7-10 nm only.

Admitting that such nuclear formations represent a universal component (especially in the meristematic cells) (Hyde 1967) and that in some cells their presence is conditioned by certain stimulating factors (Vagner-Capodano et al. 1978), and considering that their structure and chemical-molecular composition is the same, there should not exist the structural differences shown above, regardless of whether the data are obtained from related or unrelated species. We consider that these differences are due to some little technical and interpretative inadvertences.

Cytochemical data obtained by certain authors (Sankaranarayanan and Hyde 1965, Vagner-Capodano et al. 1978) prove a ribonucleoproteic composition of the loose nuclear bodies. These data are also supported by the investigations of Jordan and Chapman (1971, 1973) and those of Vagner-Capodano et al. (1978), which demonstrated an increase in the rRNA synthesis in the presence of the nuclear bodies.

Our data, as well as those of the authors mentioned above, support the idea of a nucleolar origin of the loose nuclear bodies. In all cases, a close relationship between these formations and the nucleolus was signaled. We wish to add another argument in this respect: the fibrils described by us are 16 Å in diameter, whereas Recher et al. (1969) reported that the nucleonema fibrils are 15 Å in diameter. We suppose that both the fibrils and the fibers of the loose nuclear bodies are of nucleolar origin, probably double aggregated molecular strands of rRNA: simple, at the level of the fibrils and complex at the level of the fibers.

We have already mentioned that some authors consider the presence of the nuclear bodies in the nucleus as a result of the action of certain stimulating hormones which produce a cellular hyperactivity (Recher et al. 1976, Vagner-Capodano et al. 1978). Our work confirms this point of view, the loose nuclear bodies being more frequently observed in calluses and leaflets from calluses grown in culture media containing procaine or β-naphtoxiacetic acid. The measurements performed on nucleoli also indicate that they are significantly larger in cells grown on media provided with the two substances, as compared to the control, grown on the simple (basic) medium, without growth hormones.

Correlating our data with those of other authors (Lafontaine 1965, Sankaranarayanan and Hyde 1965, Vagner-Capodano et al. 1978) which have observed loose nuclear bodies either near the nucleolus or free in the nucleoplasm and near the nucleus membrane, we support the idea that these formations are involved in the nucleus-cytoplasm transfer.

Along with the nuclear bodies, several authors have reported the presence in the nucleus, near the nucleolus, of microspherical and microcylindrical formations similar to viral structures (Sjolund and Shih 1970, Wergin et al. 1970, Jordan 1976, Mihăiescu and Mișcalençu 1979). We have not observed such structures in our electronmicrographs and consider the tissue cultures from which our material was taken as free of viruses. On the basis of these data we consider that there is no obligato-
ry relation between the loose nuclear bodies and viruses. The simultaneous existence of the two formations in the nucleus can be explained by a viral infection, independent of the existence or nonexistence of the nuclear bodies described by us and by other authors.

Figs. 5-6. 5, nucleolus disposed near nuclear membrane, presenting simultaneously both formations: loose electrondense body and unelectrondense oval area. Control meristematic callus. ×49,000. 6, three unelectrondense circular areas disposed at the periphery of the nucleus and surrounded by a layer of heterochromatin. Carnation callus after treatment with β-naphtoxialacetic acid (0.1 mg/l). ×34,000.
The ultrastructural examination of the callus material gave us the opportunity to also observe a second type of nuclear formation, either simultaneously with or in absence of the loose nuclear bodies (Figs. 4, 5, 6). This formation differs from the loose electrondense body both by the lack of electrondensity and by its weak structural expression. Also, as opposed to the loose nuclear body, which in most cases is alone in the nucleus, these unelectron-dense areas are almost always from 2 to 4 on a section surface of the nucleus. They are oval-spherical with a diameter between 0.4 and 1 µm.

There is no doubt that the unelectron-dense areas originate in the nucleolus (Figs. 4, 5) from where they later migrate to different parts of the nucleoplasm to localize, preferentially, at the periphery of the nucleus, near the nuclear membrane (Figs. 4, 6). Here they are surrounded by a layer of heterochromatin, being clearly delimited (Fig. 6).

We are not sure about the internal structure of these formations but it appears that they are composed from a mixture of fibrilar and granular material, similar to the surrounding nucleoplasmic material.

This kind of electrondense areas, with such a dispersion between the nucleolus and the nuclear membrane, have not been described until present. They are similar to the fibrillar centers (Recher et al. 1969) and to the nucleolar organizers (Jordan and Chapman 1973), but their functional behaviour in the nucleus is different. While these two nucleolar formations move from the periphery toward the interior of the nucleolus and then disperse during the increase in the activity of rRNA synthesis, the unelectron-dense areas described by us, on the contrary, get off the nucleolus and migrate toward the periphery of the nucleus.

The functional significance of this relation between the nucleolus and the nuclear membrane observed in the case of these unelectron-dense areas, must be that of an intense transfer activity (probably of rRNA) from the nucleus into the cytoplasm, as was suggested by other authors (Lafontaine 1965, Sankaranarayanan and Hyde 1965, Jordan and Chapman 1973, Vagner-Capodano et al. 1978) which showed that following the stimulation of the physiological activity of the cells there was an increase in the rRNA synthesis and an intense process of nucleus-cytoplasm transfer.

Summary

Electron microscopic investigations on the cells obtained from tissue cultures of carnation (Dianthus caryophyllus var. Linda), have demonstrated the presence of two types of particular formations in the nucleus: a) electrondense loose bodies, composed of fibrillar structures; b) unelectron-dense spherical-oval areas, with a slightly expressed structure. The possible connection of these structures with the nucleolus and nucleus-membrane functions, as well as their role in the rRNA synthesis and in the transfer processes through nucleus and cytoplasm, are discussed.

References