On the Origin of the Protein Yolk in the Oocytes of *Aplysia depilans* (Gastropoda, Opistobranchia)

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Summary. In the oocytes of *Aplysia depilans* it has been observed electronmicroscopically that 'gemmae' or 'daughter-nucleoli' separate from the nucleolus. These 'gemmae' after having overcome the nuclear membrane, would aggregate together, even after transformation, into yolk globules. These observations seem to give plausibility to the hypothesis that there is a close relationship between production of ribonucleoproteic material by the nucleolus and yolk production.

From the researches[1] carried out at this Institute of Zoology, especially on the oocytes of Molluscs and Echinoderms, the hypothesis that the protein yolk globules are formed by the aggregation of ribonucleoprotein granulations coming from the nucleus has appeared ever more convincing. The facts which have, above all, led in this direction are the following: 1. A progressive concentration of granulations, already scattered everywhere and responsible for a diffuse basophilia, into ever more dense globular formations; 2. the presence of occasional inclusions in the globules of various cytoplasmatic material (lipid drops, fragments of cytomembranes, mitochondria, etc.); 3. when the aggregations have taken place, the scarsity or absence of those granulations which were at first scattered everywhere in the cytoplasm. Attention is also drawn to the fact that considerable cytochemical research[2] has enabled us to affirm the presence of RNA in the yolk globules at the beginning of their formation. Other investigations[3], carried out especially on the oocytes of *Aplysia depilans*, have established that the yolk globules once formed then undergo a complete transformation; the definitive ones, which form the true reserve substance of the egg, have stainable affinity, ultra-structural and cytochemical characteristics different from those of the globules at the beginning of their formation. This research has also established[4] the existence in the nucleolus of 2 successive phases of activity relative to the yolk formation: the first, when it supplies the ribonucleoproteins for the initial yolk globules formation, and the second, when it supplies other substances, not yet identified, for the processes of the transformation of the yolk.

In pursuing the investigations, using also the electron microscope (pre-fixation in glutaric aldeyde at 4%, fixation in OsO_4 at 1% on a Millonig buffer; inclusion in

[1] A. BOLOGNARI, Ricerche sul nucleolo, la vitellogenesi, l'apparato del Golgi e la cellula dei tumori effettuate nell'Istituto di Zoologia dell'Università di Messina dal 1953 al 1973 (Università degli Studi, Messina, 1974).

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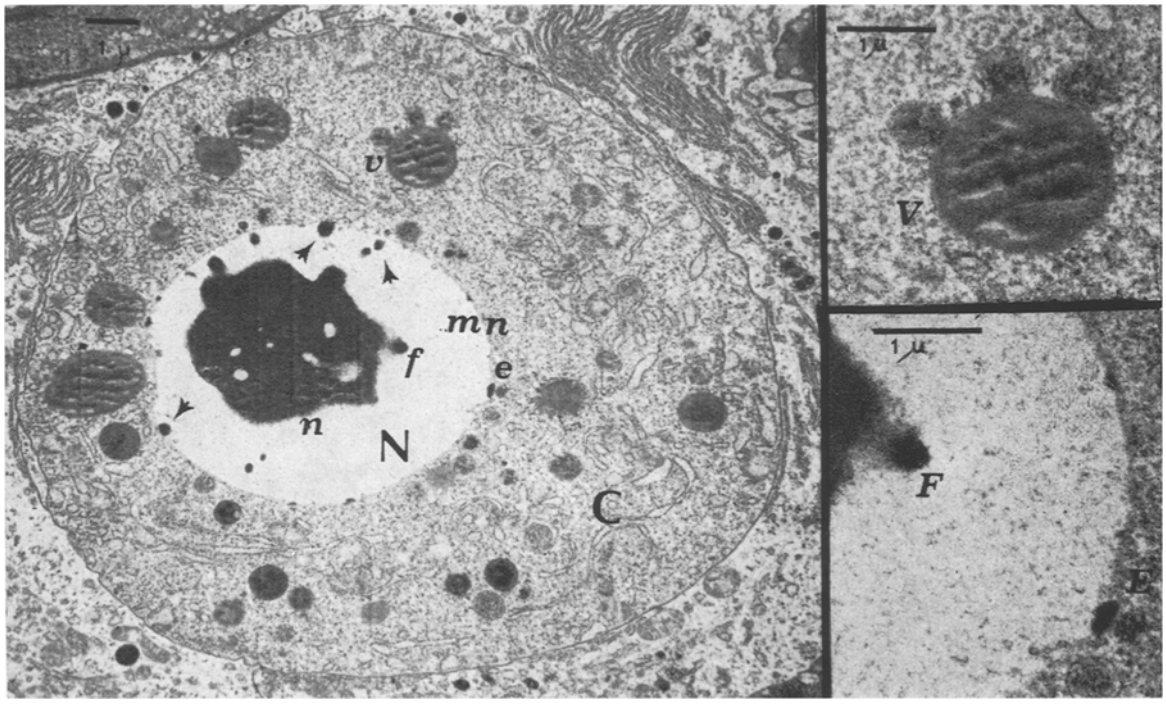
[3] A. BOLOGNARI and A. LICATA, Riv. Biol. norm. Pat. *1*, 17 (1975).

[4] P. ALBANESE, A. BOLOGNARI and M. DE SIMONE, Caryologia *16*, 57 (1963).



Oocyte of *Aplysia depilans* at the beginning of yolk production; in the nucleus (*N*) it is possible to observe that 'gemmae' of 'daughter-nucleoli' separate themselves from the nucleolus (*n*). These appear to migrate until they come in contact with the nuclear membrane (*mn*), through which they seem to transfer themselves into the cytoplasm (*C*). In this there are yolk globules in formation; to one globule there seems to adhere 3 small globules (*v*) which could have come from the nucleus. In the insert on the right upper portion, one can see the yolk globules (*V*) with 3 small globules adherring to it. In the insert on the right lower corner one can see a 'gemma' (*F*) in the act of separating itself from the nucleolus, and a point (*E*) of the nuclear membrane in which there seems to be an extrusion. Technique outlined in the text. ×2,600.

araldite-epon, contrast with uranyle acetate at 5% and with lead citrate according to Reynold), one can now have demonstrative pictures regarding the participation of the nucleolus in the initial processes of yolk formation. The Figure shows that from the nucleolus 'gemmae' or 'daughter-nucleoli' separate. These transfer themselves into the nuclear membrane. Both the surface of the nucleolus, completely irregular because of various offshoots, and its internal part because of the cavities of various forms and sizes, appear to indicate a notable activity both in the production of material and in its emission. This is especially clear at point *f* (in the insert *F*) where there is a picture of the separation of one of these 'gemmae' from the nucleolar surface. This picture has a strange resemblance to that which concerns the separation of certain forms of virus from the plasmatic membrane. At the level of the nuclear membrane, on the side of the nucleus, the 'gemmae' seem to retain their initial density; while, on the side of the cytoplasm (admitting that extrusion really takes place), they appear to have a lesser density. This is fairly clear in point *e* (in the insert *E*). In the cytoplasm there are also present various globules at the beginning of their formation. One of these (*v*, in the insert *V*) has 3 other small globules adherring to it almost as if they would come together into a larger one. They have the peculiarity of having the same ultra-structural appearance and the same density as the other globules close to the nuclear membrane. It cannot, however, be excluded that between the small globules and the larger ones there exists only a simple adhesion without this being the beginning of a fusion of the respective masses.

These new observations seem to give plausibility to the hypothesis, advanced a long time ago[1], that there is a close relationship between the production of the ribonucleoproteic material by the nucleolus and yolk production, in the sense that the former would emit, under the form of 'gemmae', the same material. The 'gemmae', after having overcome the nuclear membrane, would aggregate together, even after transformation, into yolk globules.

Application of the 'Low-Temperature Plasma Ashing Method for Biological Tissues' to Studies in the Field of Virology

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Summary. It was found that the behavior of a virus in host plant is reflected in the pattern of crystalline inorganic components of the host plant by the technique of the 'low-temperature plasma ashing method for biological tissues.'

Detailed examinations were made on the pattern of calcium oxalate crystals in the leaves of plants by comparing the pattern in the leaves affected by a virus with that in healthy leaves of the same kind, and by changing the combination of the virus and host plants. The viruses used were cucumber mosaic virus (CMV), cymbidium mosaic virus (CyMV), dendrobium mosaic virus (DeMV), and coltsfoot mosaic virus, and host plants were *Nicotiana tabacum* Linn., *N. rustica* Linn., *Dendrobium moniliforme* (Linn.) Sw., and *Beta vulgaris* var. *cicla*. Analysis of crystal pattern was made by the 'low-temperature plasma ashing method for biological tissues'[1,2] that the author had devised, with which biological tissues are completely ashed at low temperature by very reactive oxygen stream excited in a high-frequency electromagnetic field under a low pressure, preserving mineral microstructures precisely identical with the original tissue matrices.

When virus was limited to one kind and host plant varied: The cucumber mosaic virus was used, with *N. tabacum* Linn. and *N. rustica* Linn. as the host plants. These plants contain both crystal sand colony and crystal sand.

1. *N. tabacum* Linn. The main crystals distributed in healthy leaves are approximately globular crystal sand colonies (Figure A) but those in virally affected leaves are crystal sand colonies of a considerably complex shape, as shown in Figure A'.

2. *N. rustica* Linn. Crystal sand colonies are few in healthy leaves (Figure B) but are present in a considerable number in the affected leaves (Figure B'). Density of crystal sand distribution was the same as that in *N. tabacum* Linn., being higher in affected leaves than in healthy leaves.

When host plant was limited to one kind and viruses changed: The host plant used was *D. moniliforme* (Linn.) Sw., which contains both bundles of calcium oxalate raphides and crystal sand, and the viruses used were CyMV and DeMV.

1. CyMV. Bundles of raphides measuring 180–200 μm in length are found in healthy leaves (Figure C) and such crystals become 2–4 times longer in the affected leaves (Figure C'). The number of bundles of raphides tends to increase in affected leaves. This tendency becomes more marked in the distribution density of crystal sand.

2. DeMV. Infection with virus had hardly any affect on the length of bundles of raphides in the leaves and on their distribution density, but the number of crystal sand tended to increase in affected leaves.

B. vulgaris var. *cicla*, which contains crystal sand colonies, was used as the host plant and its leaves were infected with coltsfoot mosaic virus. In this case, crystal sand colony was generally smaller than those in healthy leaves (Figure D), and the size of colonies in the affected leaves (Figure D') varied considerably, with shape becoming more complex. Distribution of crystal sand colonies tended to be higher in affected leaves.

It is quite clear from the foregoing results that the behavior of viruses in the host plant is reflected in the pattern of crystalline inorganic components in the host plant. In addition, the following conclusion may be drawn from the results of these observations: 1. The morphological principle of the crystals contained in the plants used in the present series of experiments remains unchanged even when the plant itself undergoes marked biochemical and morphological alterations. This fact can be applied to other plants with a considerably high probability. 2. When a plant possesses a pattern of

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[2] K. Umemoto, Chem. pharm. Bull., Tokyo *19*, 217 (1971); Yaku gaku Zasshi (J. pharm. Soc. Jap.) *91*, 828 (1971).