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SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

Genetica. — *Cytological localization of molecular hybrids between rRNA and DNA in the embryo suspensor cells of Phaseolus coccineus. A preliminary note* (*). Nota di SILVANA AVANZI, MARIO BUONGIORNO-NARDELLI, PIER GIORGIO CIONINI e FRANCESCO D'AMATO, presentata (**) dal Corrisp. F. D'AMATO.

RIASSUNTO. — RNA ribosomale (rRNA) triziato di *Vicia faba* è stato ibridato con il DNA di sezioni di cellule del sospensore dell'embrione di *Phaseolus coccineus*. È stato trovato che le cellule del sospensore – sia quelle con basso o medio grado di endopoliploidia sia quelle con cromosomi politenici – presentano amplificazione dei cistroni (rDNA) che codificano la sintesi dell'rRNA.

Nelle cellule a basso o medio grado di endopoliploidia l'ibridazione DNA–rRNA (marcatura) si manifesta a livello delle regioni nucleolari.

Nelle cellule a cromosomi politenici, la marcatura si ha non solo negli organizzatori, ma anche in altre regioni eterocromatiche al difuori del sistema di organizzazione del nucleolo. La maggioranza dei *DNA puffs* non sono marcati (ciò indica che altri geni, che non sono rDNA, possono subire amplificazione). Tra gli *RNA puffs* osservati due hanno mostrato alcuni grani di argento.

INTRODUCTION

In *Phaseolus coccineus* and *P. vulgaris*, Nagl [18, 19, 24] has shown that the suspensor originates from the basal cells of the proembryo, which undergo chromosome endoreduplication. In the full grown typically club-shaped suspensor, the degree of endopolyploidy progressively rises towards its distal (micropylar) portion, in which giant cells with polytene chromosomes occur. The maximum degree of endopolyploidy attained by these cells is estimated to be 4096 x. In the giant cells, the polytene chromosomes are present in the diploid number ($2n = 22$) and the homologues are not paired. Because of their characteristic lengths and pattern of distribution of euchromatic and heterochromatic regions, several polytene chromosome pairs can be recognized; the most easily identified are the two nucleolus-organizing pairs [20, 21]. In material grown at different temperatures or subjected to actinomycin D treatment, Nagl [22, 23, 24] has also described *a.o.*: i) the behaviour of the nucleolar puff and of the intranucleolar chromatin, ii) the induction of

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(**) Nella seduta del 13 marzo 1971.

a lampbrush state in the polytene chromosomes, iii) the extrusion of material from the nucleolus, or nucleoli, and the formation of additional nucleoli.

More recently, Avanzi and coll. [2] have studied the suspensor cells of *P. coccineus* by means of specific staining techniques (Feulgen, methyl green-pyronin, toluidine blue-molybdate, acridine orange in fluorescence microscopy), DNase and RNase digestion tests and autoradiographic techniques (incorporation of tritiated thymidine, uridine, lysine, tryptophan; DNA detection by means of tritiated actinomycin D). This study has shown, *a.o.*, that:

i) the cells of the suspensor—both those with low or medium degree of endopolyploidy (which form the " handle " portion of the club-shaped suspensor: fig. 1, *h*) and those with polytene chromosomes (which form the " knob " portion of the suspensor: fig. 1, *k*)—undergo extra DNA synthesis. In polytene chromosome cells, extra DNA synthesis can be easily localized: it occurs not only in the nucleolus organizing system (organizers and satellites) but also at many heterochromatic regions scattered throughout the chromosome complement;

ii) from the regions of extra DNA synthesis, micronucleoli (Nagl's additional nucleoli) are released into the nucleoplasm and from here into the cytoplasm. Micronucleoli are composed of a spherical mass of ribonucleoprotein covered by a layer of DNA and traversed, sometimes at least, by thin DNA strands;

iii) the extra DNA codes for RNA when still attached to the chromosome;

iv) the extra DNA—or part of it—is complexed with histone.

The phenomena observed in *P. coccineus* are similar to those occurring in the oöcytes of amphibia [4, 8, 10, 11] and insects [12, 16] in which amplification of the cistrons (rDNA) coding for ribosomal RNA (rRNA) has been demonstrated.

In the last two years, techniques have been developed for DNA–RNA hybridization in cytological preparations: they have led to the microscopical localization of ribosomal cistrons in the oöcytes of *Xenopus* [13, 15], HeLa cells [15], chinese hamster cells [6] and dipteran polytene chromosome cells [25].

In this note, we report on a preliminary experiment in which rRNA of *Vicia faba* has been hybridized with DNA in sectioned cells of the suspensor of *P. coccineus*.

Materials and methods

Developing seeds of *Phaseolus coccineus* were collected after reaching a size which, according to previous experience, corresponds to a stage when the cotyledons fill one third to one half of the endospermatic cavity. The seeds were fixed for 3 h in absolute ethanol-chloroform 2 : 1 (v/v), embedded

in paraffin and sectioned at 6 μm. After removal of paraffin and hydration, the sections were collected on membrane filters (Millipore GS type, pore size 0.2 μm).

Vicia faba rRNA was labelled to a high specific activity (100,000 d.p.m/μg) by feeding growing seedlings with ^{3}H–5–uridine (Radiochemical Centre, Amersham, 23 Ci/m mole) at 35 μCi/ml for 4 days; a chase with non-radioactive uridine for 6 hours followed. Ribosomal RNA was extracted from the tissues and purified by sucrose gradient centrifugation [1].

For DNA–RNA hybridization, the procedure described by Buongiorno-Nardelli and Amaldi [6] was used. Denaturation of DNA was carried out by heating the tissue sections on filters in SSC/10 at 100° C for 10 min. (SSC = = 0.15 M NaCl—0.015 M sodium citrate). Annealing was then performed at 65° C for 15 h in 2×SSC containing 3 μg/ml of the ^{3}H—rRNA of *Vicia faba*. From then on the sections on filters were treated for conventional DNA–RNA hybridization [14]. The specimens were thoroughly washed by filtration with 2×SSC and then treated with RNase (10 μg/ml) for 1 h at room temperature in 2×SSC. After another washing, by filtration in 6×SSC, the sections were air-dried and glued on slides. The slides were dipped in undiluted Kodak NTB 2 emulsion, air-dried and exposed in a dry atmosphere at 0–4° C for 90 days. The slides were processed with Kodak D–19 developer and stained with haematoxylin. The membrane filters were clarified with immersion oil.

Sections of suspensors were also used for localization of DNA with a very sensitive method: the ^{3}H–actinomycin D DNA–binding technique [3, 7, 9] according to the method previously used by Avanzi and coll. [2].

RESULTS AND DISCUSSION

Ribosomal RNA of *Vicia faba* has been shown to hybridize with the DNA in cytological preparations of *P. coccineus*; this result could be expected because *Vicia* and *Phaseolus* belong to the same family (Leguminosae) and it is known that the rRNA of several eukaryotes will cross-hybridize with the DNA of other eukaryotic species [5].

For our DNA–RNA hybridization experiments, we have used longitudinal sections of ovules which included the whole suspensor and have studied three suspensors in different developmental stages, as indicated by the size of the embryo cotyledons. In most nuclei of the cells forming the " handle " portion of the suspensor (fig. 1, *h*), the nucleolar regions were labelled; this situation was clearly in contrast with that of the embryonic cells which never showed labelling.

In the polytene chromosome cells (" knob " portion of the suspensor: fig. 1, *k*), it was observed that:

i) in the nucleolar regions (polytene chromosome cells generally contain a large irregularly shaped nucleolus organized by the two satellite chromosome pairs) the organizers were labelled (figs. 2, 4, 5). The nucleolar body was

generally unlabelled; sometimes a sparse distribution of silver grains within the nucleolar body was observed;

ii) some heterochromatic regions outside of the nucleolus organizing system were labelled (figs. 2, 3);

iii) among the RNA puffs observed, two showed silver grains (figs. 6 and 7).

The present observations show that amplification of ribosomal cistrons occurs in the cells of the embryo suspensor of *P. coccineus*, both those with low and medium degree of endopolyploidy and those with polytene chromosomes. The DNA–RNA hybridization also shows that ribosomal cistrons are present not only in the nucleolus organizing system, but also in other regions of the genome of *P. coccineus*. This finding conforms with the previous observations of Avanzi and coll. [2] on extra DNA synthesis in *P. coccineus* referred to in the Introduction.

If it is accepted that actinomycin D binds selectively to guanine [17], the behaviour of ^{3}H–actinomycin D as a DNA " stain " can give an indication that the rDNA of *P. coccineus* is rich in C–G. In fact, we have seen that the nucleolus organizers in *P. coccineus* are the first to appear labelled after short exposures and, at longer exposures, are always clearly more labelled than most of the genome. Only some chromosome segments behave similarly to nucleolus organizers towards ^{3}H–actinomycin D binding.

Since we have seen no hybridization over most of the DNA puffs in *P. coccineus*, we can conclude that genes other than rDNA can undergo amplification. In this connection, we recall that the DNA puffs of *Rhynchosciara hollaenderi* do not hybridize with rRNA [25].

Work on DNA–RNA hybridization in *P. coccineus*, including competition experiments, is being continued.

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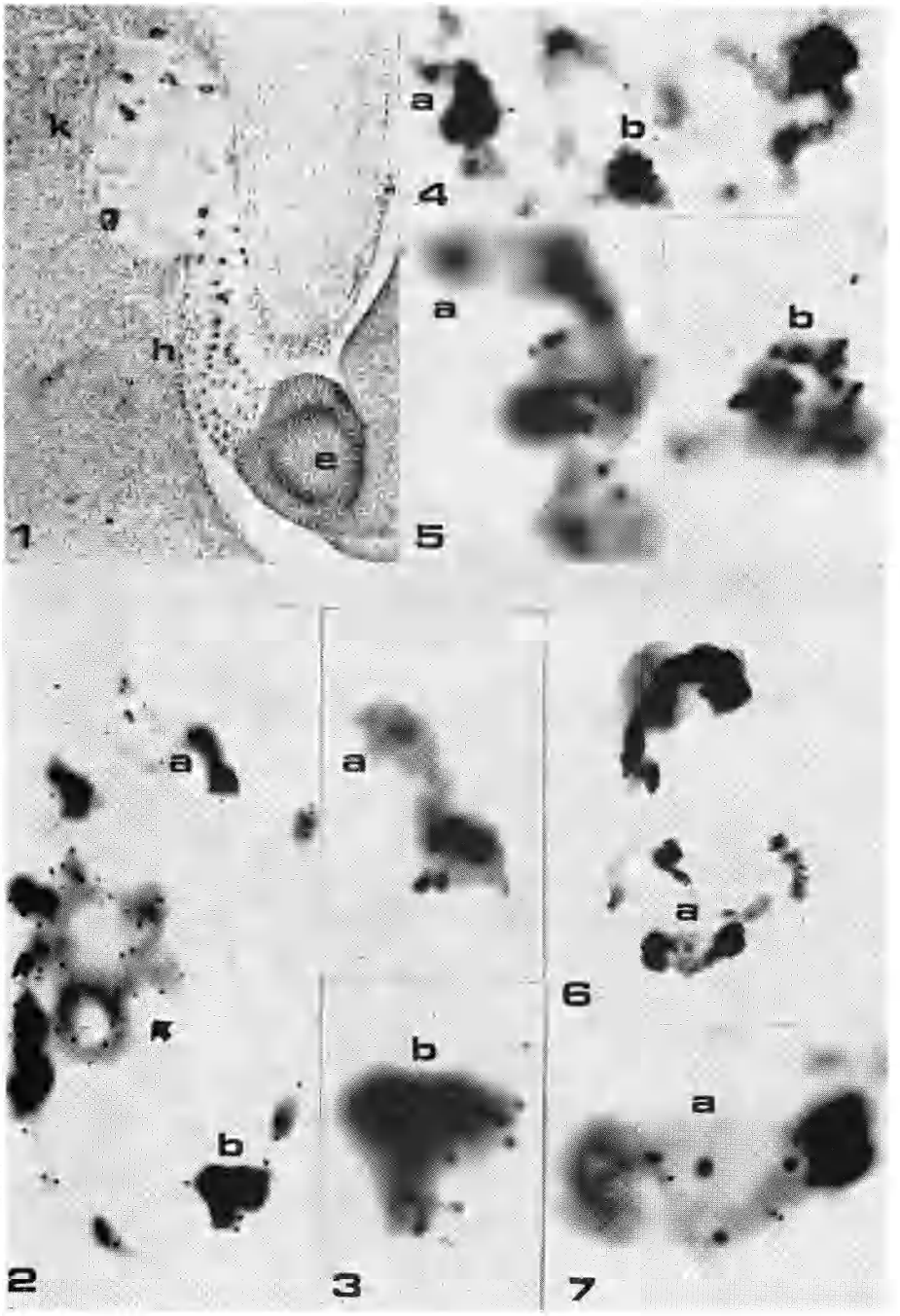
References

[1] G. Attardi, H. Parnas, M. I. H. Wang and B. Attardi, « J. Mol. Biol. », *20*, 145 (1966).
[2] S. Avanzi, P. G. Cionini and F. D'Amato, « Caryologia », *23*, 605 (1970).
[3] J. Brachet and A. Ficq, « Exptl. Cell Res. », *38*, 153 (1965).
[4] D. D. Brown and I. B. Dawid, « Science », *160*, 272 (1968).
[5] D. D. Brown, C. S. Weber and J. H. Sinclair, « Carnegie Inst., Year Book », *66*, 580 (1967).
[6] M. Buongiorno-Nardelli and F. Amaldi, « Nature », *225*, 946 (1970).
[7] B. S. Ebstein, « J. Cell. Biol. », *35*, 709 (1967).
[8] D. Evans and M. Birnstiel, « Biochim. Biophys. Acta », *166*, 274 (1964).
[9] A. Ficq, « Exptl. Cell Res. », *53*, 691 (1968).
[10] J. G. Gall, « P.N.A.S. », *60*, 553 (1968).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[11] J. G. GALL, « Genetics » Suppl. *61*, 121 (1969).
[12] J. G. GALL, H. C. MacGREGOR and M. E. KIDSTON, « Chromosoma », *26*, 169 (1969).
[13] J. G. GALL and M. L. PARDUE, « P.N.A.S. », *63*, 378 (1969).
[14] D. GILLESPIE and S. SPIEGELMANN, « J. Mol. Biol. », *12*, 829 (1965).
[15] H. A. JOHN, M. BIRNSTIEL and K. W. JONES, « Nature », *223*, 582 (1969).
[16] A. LIMA-DE-FARIA, M. BIRNSTIEL and H. JAWORSKA, « Genetics », Suppl. *61*, 145 (1969).
[17] W. MÜLLER and D. M. CROTHERS, « J. Mol. Biol. », *35*, 251 (1968).
[18] W. NAGL, « Öster. Bot. Z. », *109*, 431 (1962).
[19] W. NAGL, « Naturwiss. », *49*, 261 (1962).
[20] W. NAGL, « Chromosoma », *16*, 511 (1965).
[21] W. NAGL, « Öster. Bot. Z. », *114*, 171 (1967).
[22] W. NAGL, « Naturwiss. », *56*, 221 (1969).
[23] W. NAGL, « Chromosoma », *28*, 85 (1969).
[24] W. NAGL, « J. Cell. Sci. », *6*, 87 (1970).
[25] M. L. PARDUE, S. A. GERBI, R. A. ECKHARDT and J. G. GALL, « Chromosoma », *29*, 268 (1970).

EXPLANATION OF PLATE I

Figs. 1–7. – Embryo suspensor of *Phaseolus coccineus*. Magnification: fig. 1, ×44; figs. 2, 4 and 6, ×1200; figs. 3, 5 and 7, ×3400.

Fig. 1. – Longitudinal section of ovule showing part of the embryo (*e*) and the club-shaped suspensor with its « handle » (*h*) and « knob » (*k*) portion.

Fig. 2. – Portion of a polytene chromosome nucleus showing DNA–rRNA hybridization at the organizers attached to a vacuolated nucleolus (arrow) and at two chromosome regions (*a*, *b*) not belonging to the nucleolus organizing system. Fig. 3. Details of fig. 2 at higher focus and greater magnification.

Fig. 4. – Portion of a polytene chromosome nucleus with DNA–rRNA hybridization at two organizers (*a*, *b*); the nucleolus is not seen, being contained in other sections. Fig. 5. Details of fig. 4 at higher focus and greater magnification.

Fig. 6. – Portion of a polytene chromosome nucleus showing silver grains over an RNA puff. Fig. 7. The RNA puff of fig. 6 at higher focus and greater magnification.