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Satellite-Rich DNA in Cucumber: Hormonal Enhancement of Synthesis and Subcellular Identification

(chloroplast DNA/mitochondrial DNA/gibberellin/hypocotyl elongation)

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ABSTRACT Cucumber hypocotyl DNA in neutral CsCl distributes into a mainband comprising 59% of the total, and two large satellite bands which contribute 41% to the DNA pattern. Organelle enrichment studies show that the densities of mitochondrial and chloroplast DNA coincide with those of the satellite bands. At least 12–19% of total cucumber DNA is associated with the cytoplasmic organelles. These values, which are several times larger than those usually quoted for higher plants, are correlated with an unusually low amount of DNA per haploid nucleus in cucumber. Synthesis of the satellite DNAs, as well as mainband DNA, is appreciably stimulated *in vivo* by application of the plant hormone, gibberellin. Endogenous and hormone-enhanced synthesis of the satellite DNAs is proportionately greater in target tissue showing a high rate of organelle synthetic activity.

In prior studies, gibberellin-induced elongation of the cucumber hypocotyl was correlated with an increased incorporation of [^{3}H]thymidine into DNA (1). Autoradiographic and fractionation experiments (2) further suggested that much of this synthesis might be extranuclear, reflecting a hormone-enhanced multiplication of chloroplasts in the elongating zone (3). This possibility has now been investigated at the level of different DNA density peaks by CsCl gradient centrifugation. In this regard, several higher plants, including cucumber, have previously been shown to contain particularly large amounts (up to 44%) of satellite DNAs in neutral CsCl (4–8). These large plant satellites—in analogy with the satellite DNAs of animal cells (9)—have variously been reported to be rapidly renaturing (4, 7, 8, 10), associated with the genes for rRNA (4, 11) and of nuclear origin (7, 10, 12). The relationship between cytoplasmic organelle DNA and the large plant satellites, however, was not seriously investigated.

In the present report we demonstrate that a considerable fraction of the large satellite DNAs in cucumber are associated with chloroplasts and mitochondria. We further show that in elongating hypocotyl tissue the rates of synthesis of organelle-rich satellite DNA and mainband DNA respond *in vivo* to hormone treatments by increasing markedly.

METHODS

Plant Material. Light grown cucumber seedlings (*Cucumis sativus* L. cv. Improved Beit Alpha) were treated with gibberellin and labeled for 1 hr with [^{3}H]thymidine as described previously (2). Addition of 1000 ppm ampicillin (Beecham Research Laboratory) to the labeling solution reduced the bacterial titer on Bacto-penassay agar to $<10^5$ colonies (representing <0.001 μg of DNA) per amount of plant tissue yielding 1.0 μg of DNA. At the end of the labeling period, tissue sections were rinsed in distilled sterile water, Tris-saline-EDTA (0.15 M NaCl, 0.1 M EDTA, 0.05 M Tris·HCl, pH 8.0) was added and the material was ground with acid-washed glass powder (Fisher, 200 mesh) by mortar and pestle for DNA extraction.

Preparation of Subcellular Organelle Fractions. Ten grams of hypocotyl sections were homogenized by an automatic Tissue Chopper (Yeda Instruments no. 92,001, Rehovot, Israel) in modified Honda medium (0.25 M sucrose, 0.01 M mercaptoethanol, 0.01 M Mg acetate, 2.5% Ficoll 400,000 d, 5% dextran-T 150, 0.03 M Tris·HCl, pH 7.4) at 2–4° and were filtered through cheesecloth. Nuclei (method 1) were isolated from the filtered homogenate on a discontinuous gradient of 20, 40, and 50% sucrose as described in ref. 2. Alternatively (method 2), hypocotyl sections were processed as described by Tautvydas (17) except that steeping was carried out at 0–2° in 4% gum arabic, 0.4 M sucrose, 6 mM Mg acetate, 0.3 mM dithiothreitol, and 5 mM morpholinoethane sulfonate, pH 6.4. Resuspension buffer was without gum arabic and contained 0.15% 2-ethyl-1-hexanol. Method 3 was identical to method 2 except that 0.1% Triton X-100 was included during the steeping procedure. For the isolation of chloroplasts the filtered homogenate was centrifuged at 1000 $\times$ *g*, and the pellet was dispersed in modified Honda medium supplemented with 40 mM Mg^{+2} and incubated with DNase (70 μg/ml) at 0° for 15–20 min. After addition of 0.1 M EDTA, discontinuous sucrose gradient centrifugation was carried out (2). For the isolation of mitochondria filtered homogenate was centrifuged at 5000 $\times$ *g* for 5 min and the supernatant was recentrifuged at 23,500 $\times$ *g* for 20 min. The 23,500 $\times$ *g* pellet was treated with DNase as described for chloroplasts and then rinsed in Tris-saline-EDTA.

DNA Extraction and CsCl Density Gradient Centrifugation. Homogenates in Tris-saline-EDTA were lysed at room temperature with a final concentration of 4% sodium lauryl sarcosinate (gift from Geigy). DNA extraction was as described by Marmur (18). Polysaccharide contaminants were removed by sedimentation at 30,000 $\times$ *g* for 20 min or by carbohydrate exchange chromatography (14). Analytical CsCl density gradient centrifugation was carried out at 44,000 rpm in a Spinco model E ultracentrifuge with rotor An-D (19). Preparative density gradient centrifugation was at 40,000 rpm and 20° for 48 hr in rotor 50 (fixed angle) in a Spinco ultracentrifuge.

RESULTS

CsCl-DNA density patterns of subcellular fractions

Fig. 1 shows a typical CsCl density profile obtained for whole cell cucumber DNA extracted from the elongating zone

TABLE 1. *Relative amounts of satellite and mainband DNAs in cucumber*

	%* DNA species		
	1.707 g cm^{-3}	1.701 g cm^{-3}	1.692 g cm^{-3}
A. Whole cell preparations—selected tissues			
Elongating zone of hypocotyl	20	21	59
Apical meristem	21	20	59
Root meristem	25	16	59
B. Nuclear preparations—elongating zone			
Sucrose gradient (method 1)	10	14	76
Gum arabic gradient (method 2)	12	12	76
Triton X-100, gum arabic gradient (method 3)	17	19	64

Analytical ultracentrifugation in CsCl was performed as described in Fig. 1.

* Arrived at by least square computer analysis of the ultraviolet absorbance profiles. The averages of several preparations are shown (variation, ±1%).

The DNA separated into a main component at 1.692 g cm^{-3}, comprising 59% of the total, and two satellite bands at 1.701 and 1.707 g cm^{-3} which together contributed 41%. The density peaks of DNAs extracted from subcellular fractions enriched in nuclei, chloroplasts, and mitochondria are also shown. In the nuclear fraction the satellite DNA peaks were considerably diminished relative to the main component at 1.692 g cm^{-3}. On the other hand, DNA from the chloroplast fraction showed a major component at 1.701 g cm^{-3} and a shoulder at 1.707 g cm^{-3}, while mitochondrial fraction DNA distributed as a single peak at 1.707 g cm^{-3}. It is likely that a good part of the shoulder at 1.707 g cm^{-3} in the chloroplast fraction is due to mitochondrial contamination, particularly in light of the greater resistance exhibited by these organelles to DNase digestion (31). Plant mitochondria can also be extremely heterogeneous in size (20, 21) and may often contaminate isolated chloroplast fractions (22, 31). Taken together, our results suggest that mitochondrial and chloroplast DNAs in cucumber have distinct buoyant densities of 1.707 and 1.701 g cm^{-3}, respectively.

We noted with interest the coincidence of both cytoplasmic organelle DNA densities with those of the large satellites in the whole cell profile. If the organelle DNAs were indeed related to the satellites, we reasoned that the chloroplast-associated satellite band might be more prominent *vis-à-vis* the mitochondrial one in green tissues and vice versa in non-green tissue. The data in Table 1A suggest that this may indeed occur in the cucumber seedling. Thus, root meristems contained 19–25% more of satellite $DNA_{1.707}$, while chloroplast-rich tissues (elongating hypocotyl, apical meristem) contained 25–31% more of satellite $DNA_{1.701}$. This same trend holds for cucumber leaves (compare ref. 7).

Quantitative estimation of mitochondrial and chloroplast DNA

The contribution of mitochondrial and chloroplast DNA to the total cell pattern can be estimated from the diminution of the satellite DNAs in the nuclear fraction patterns. Table 1B summarizes the data obtained with three different methods of preparing nuclei. In each instance there is a reduction in the percentage of satellite DNAs; however with Triton X-100 the decrease (10–15%) is clearly the mildest. Nuclei prepared without detergent and under conditions where lysis of mitochondria and chloroplasts was avoided showed reductions of 40–50% for $DNA_{1.701}$, and 33–43% for $DNA_{1.701}$.

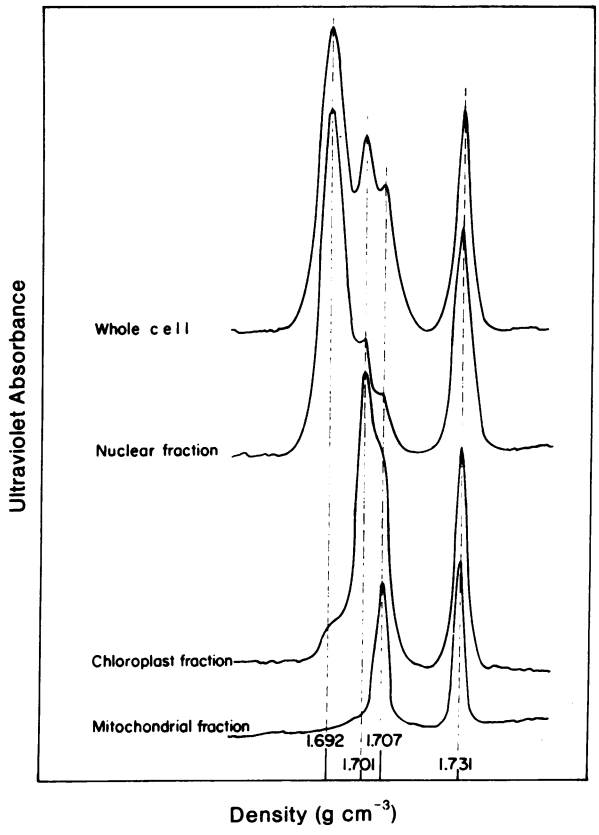

FIG. 1. Analytical CsCl density patterns of cucumber DNA extracted from whole cells and subcellular fractions. One to three micrograms of DNA were used for each determination. The nuclear fraction was prepared by method 1. Chloroplast and mitochondrial fractions were incubated with DNase prior to membrane lysis to degrade non-protected, adhering DNA molecules (22). *Micrococcus lysodeikticus* DNA (1.731 g cm^{-3}) was used as a density marker.

The high amount of non-nuclear DNA in cucumber was confirmed in experiments designed to determine, for each DNA peak, the percent resistance to DNase before lysis of the cytoplasmic organelles. For this purpose hypocotyls were labeled with [^{14}C]thymidine, homogenized, and directly in-incubated with DNase. After termination of the reaction, homogenate labeled with [^{3}H]thymidine but not treated with DNase was added, and the mixture was lysed and DNA was extracted. In parallel, a control mixture of untreated ^{14}C- and ^{3}H-labeled hypocotyls was prepared. Fig. 2 shows the radioactivity patterns obtained for both DNA mixtures after preparative CsCl density gradient centrifugation. The satellite and mainband regions are readily discernible in the control mixture and a plot of [^{14}C/^{3}H] across the gradient produced a straight line. In the mixture containing the DNase-treated sample the [^{3}H]thymidine profile, as expected, resembled that of the control; however, the ^{14}C profile showed a definite skewness towards the satellite region. By comparing the ratio of radioactivities in the control and treated mixtures, we could determine the percent of ^{14}C-labeled material resistant to DNase across the gradient. The plot in this case yielded a

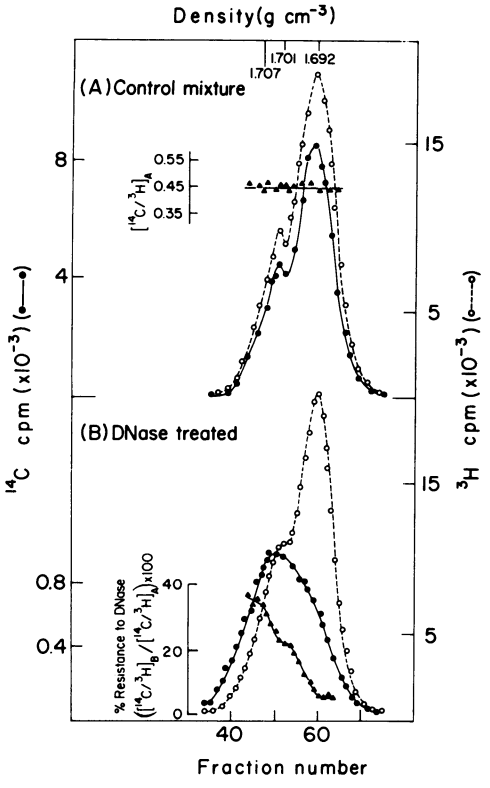

FIG. 2. CsCl DNA patterns of whole cell homogenates treated with DNase prior to membrane lysis. Seedlings were labeled with [^{3}H]- or [^{14}C]thymidine and divided into four groups. Sectioned hypocotyls were evacuated and kept for 12 hr at 0° in resuspension buffer, pH 6.8 (see preparation of nuclei, method 2). The infiltrated tissues were then homogenized, concentrated by centrifugation (30,000 × *g*, 30 min, 0°), and suspended in resuspension buffer containing 40 mM Mg^{+2}. One of the ^{14}C-labeled samples was incubated with 70 μg/ml of DNase for 20 min at 0°, the reaction being terminated by the addition of excess resuspension buffer containing 0.1 M EDTA. All other samples were mock treated. The homogenates were reconcentrated as above, suspended in Tris-saline-EDTA, and combined to produce two ^{14}C-, ^{3}H-labeled mixtures which were then lysed and DNA was extracted. Preparative CsCl density gradient centrifugation was carried out as described in *Methods*. The densities of individual fractions were calculated from refractive index measurements across the gradient while the positions of the DNA peaks were confirmed in separate analytical ultracentrifugal analyses (not shown). (A) *Control mixture*—includes mock-treated ^{14}C- and ^{3}H-labeled samples; (B) *DNase treated*—includes the ^{14}C-labeled DNase-treated sample and a ^{3}H-labeled mock-treated sample. % *Resistance to DNase*—calculated using the formula $([^{14}C/^{3}H]_B/[^{14}C/^{3}H]_A) \times 100$. ●——●, [^{14}C]thymidine; O- - -O, [^{3}H]thymidine; ▲——▲, [^{14}C/^{3}H].

curve with three plateau regions at the respective densities of the three DNA peaks; the resistance being about 35% at 1.707 g cm^{-3}, 22% at 1.701 g cm^{-3}, and 5–6% at 1.692 g cm^{-3}. Thus, a considerable portion of the satellite DNAs proved to be membrane protected [a property specifically associated with cytoplasmic organelles (15, 23, 24) rather than the nucleus], while little of the mainband DNA remained after the DNase treatment. With the values for whole cell DNA shown in Table 1A, the quantitative estimations derived from the data in Table 1B and Fig. 2 suggest that in the elongating zone from 7 to 10% of total cucumber DNA is mitochondrial

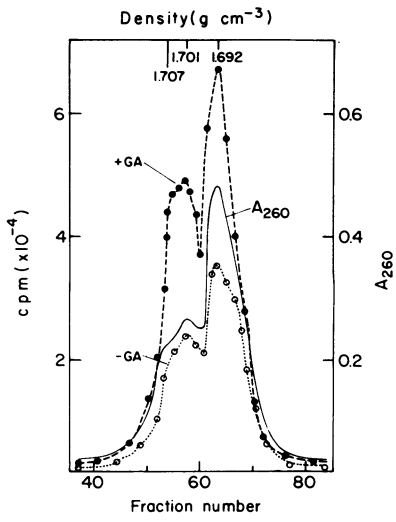

FIG. 3. CsCl density patterns of newly synthesized DNA from hormone-stimulated and normal elongating hypocotyls. Gibberellin treatment, [^{3}H]thymidine labeling, DNA extraction, and preparative ultracentrifugation are described in *Methods*. The densities of individual CsCl fractions and the positions of the DNA peaks were determined as described in Fig. 2. The two DNA samples were centrifuged simultaneously in separate tubes of the same rotor. The A_{260} patterns obtained were very similar and only that for the hormone-induced sample from the elongating zone is shown. ●- - -●, gibberellin-treated (+GA); O· · ·O, control tissue (−GA); ——, A_{260}.

while about 5–9% is chloroplastic. We take these to be minimum values, since they are necessarily based on imperfect subcellular fractionations and the assumption of minimal damage to organelle membranes during homogenization.

Normal and hormone-induced synthesis of satellite and mainband DNAs

In light of the partial correspondence shown in the preceding sections between the cytoplasmic organelle DNAs and the satellite bands, it was of interest to determine the normal and hormone-induced patterns of DNA synthesis for the various peaks generated by CsCl gradient centrifugation. In Fig. 3 the absorbance levels at 260 nm for the gibberellin-treated (+GA) and control (−GA) samples are very similar; thus the two radioactivity patterns may be directly compared. Clearly the specific radioactivities of all DNA components from the elongating zone are increased as a consequence of the hormonal treatment.

The data from several such experiments are brought together in Table 2 and compared with similar information for DNA synthesis in the meristematic zone of the same seedlings. The results may be summarized as follows: (*a*) Hormonal treatment significantly enhances [^{3}H]thymidine incorporation into all peaks of DNA in both kinds of tissue. (*b*) The extent of enhancement of the satellite DNAs is similar to that of mainband DNA. (*c*) Hormonal stimulation of DNA synthesis in the elongating zone is at least three times greater than in the meristematic zone. (*d*) In the meristematic zone there is no difference in the level of synthetic activity among the various DNAs, but in the elongating zone the rates of synthesis of the satellite DNAs are significantly higher than that of the mainband DNA. These differences in activity are

TABLE 2. *Gibberellic acid stimulation of DNA synthesis in elongating and meristematic zones of the hypocotyl*

	Elongating zone			Meristematic zone		
	DNA species			DNA species		
	Satellites (cpm × 10^{-2}/μg of DNA)	Mainband	Sat/ Main	Satellites (cpm × 10^{-2}/μg of DNA)	Mainband	Sat/ Main
Control	16.6	11.2	1.48	14.3	14.6	0.98
Hormone-treated	33.6	22.2	1.51	18.4	18.4	1.00
% Stimulation	102	98		28	26	

Incorporation of [^{3}H]thymidine into different DNA components was determined by preparative CsCl density gradient centrifugation as in Fig. 3. Elongating and meristematic zones were taken from the same seedlings. The data are from one of several experiments which were carried out. Specific radioactivities were calculated for the total areas under the mainband and combined satellite region. The derived values (Sat/Main; % stimulation) varied by $<\pm 5\%$ in the different experiments.

maintained proportionately also after the external, hormone-induced boost of synthesis.

DISCUSSION

The elongating hypocotyl of untreated cucumber seedlings differs from the meristematic region by showing a greater responsiveness to hormonal stimulation at the level of DNA synthesis, and a bias in the rate of this synthesis in favor of the satellite DNAs. These findings are in agreement with other observations which show that the morphological effect of gibberellin treatment—i.e., elongation—is considerably more pronounced in this zone than in the meristematic one and is associated with a greater increase in chloroplast number per cell (ref. 3; and to be published). It is enticing to speculate that these differences in response between tissues may be caused by endogenous gibberellin which is found in cucumber seedlings (26). From our studies it is not yet clear whether the increase in nucleic acid synthesis that occurs in the elongating hypocotyl cells as a result of hormonal stimulation is a manifestation of, or prerequisite for, the morphological growth response.

Another observation of particular interest is that within a specific tissue the hormone stimulates synthesis of mainband and satellite DNAs to the same extent. Insofar as the latter are related to organelle DNA, it suggests that gibberellin may be acting on target molecules located at some common site within the cell (presumably an extra-organelle component). This hypothesis is compatible with current studies showing regulation of mitochondrial and chloroplast biogenesis to involve cooperation of nuclear and organelle genomes (27). Apart from our own initial work with gibberellins in the cucumber system (2, 25), the effects of thyroid and adrenocortical hormones on RNA and protein synthesis in animal mitochondria have been investigated (compare ref. 16). Few other reports exist in this sphere; however, it is already becoming apparent that in order to interpret the effects of hormones on nucleic acid synthesis within target cells, the possible responses of the organelle genomes in the cytoplasm must be taken into account.

Previous studies dealing with cucumber DNA (6, 7) have relegated the satellite bands to the nucleus essentially on the basis of three arguments: (*a*) The buoyant density often cited for several higher plant chloroplast DNAs did not match that of either cucumber satellite. (*b*) Mitochondrial DNA represents such a small percentage of the total that it cannot be seen in analyses of total DNA. (*c*) Nuclear fractions, treated with Triton X-100 to solubilize the cell organelles, still showed the satellite DNAs. The conclusions arrived at in the present communication are in obvious disagreement with the above. We offer the following comments: (*a*) The identification of chloroplast DNA in higher plants has been the subject of many conflicting reports (compare refs. 28 and 29). Attempts at ordering the confusion in the literature have recently been made (30, 31), resulting in the view that higher plant chloroplast DNAs band at 1.696 ± 0.001 g cm^{-3} in neutral CsCl. However, this generalization may be too rigid to encompass all of the angiosperms (29). The extensive studies on chloroplast DNA from tobacco (13, 32) as well as our own results with cucumber suggest that other buoyant densities (in these cases, 1.701 ± 0.001 g cm^{-3}) are also valid for chloroplast DNAs from some higher plants. (*b*) The purportedly low percentage of mitochondrial DNA in cucumber is by way of generalization only (7); direct quantitative measurements do not appear to have been carried out previously. (*c*) Solubilization of cytoplasmic organelles by Triton X-100 does not, in itself, assure the purity of nuclear DNA in subcellular fractions. The situation in cucumber (where >40% of the DNA is satellites) may be analogous to the contamination of mitochondrial fractions with nuclear DNA in preparations containing ruptured nuclei (15, 23). In such cases maintaining nuclear integrity is one way of eventually reducing cross contamination of DNAs (13, 33).

Our results suggest that, at a minimum, 12–19% of cucumber DNA is located within the cytoplasmic organelles. These values are several times those usually quoted for higher plants. In attempting to understand this disparity special note should be taken of the fact that *Cucumis sativum* has a DNA content per haploid nucleus of 0.5 to 0.9 pg (12, 34). These values are among the very lowest for more than 800 angiosperms investigated (35) and are 15 and 90 times lower than the average haploid nuclear DNA contents determined for more than 200 dicots and monocots, respectively (36). Since the size (1.5–4.5 μm average diameter) and number (15–30/cell) of chloroplasts in elongating cucumber hypocotyl appear to be average (Kadouri and Atsmon, unpublished), the disproportionately low nuclear DNA content in this organism may explain the relatively high proportion of organelle DNA. Interestingly, a search of the literature has revealed several other dicots in which a particularly high amount of satellite band DNA is associated with a very low amount of nuclear DNA (Compare data in ref. 7 with 12 and 36). Included in this group is *Antirrinum majus*, whose

satellite DNA band (>20% of the total) has recently been equated with chloroplast DNA (24). Similarly, *Nicotiana tabacum* has a very low nuclear DNA content (36) and a high level of chloroplast DNA (13).

The inverse relationship described above does not exclude the possibility that the large plant satellites may be complex mixtures of several types of DNA (compare ref. 8). Indeed, in some plants, sequences complementary to ribosomal RNA appear to be concentrated in the satellite band (4, 10). These have been estimated to account for perhaps 15% of the satellite in muskmelon (8), and to contain cistrons for both cytoplasmic and chloroplastic rRNAs in pumpkin (11). Other rapidly reannealing and repetitive satellite DNA sequences whose functions were not determined have also been described (7, 8). While these have been considered to represent nuclear satellite DNAs we wish to point out that DNAs from cytoplasmic organelles exhibit very similar reannealing properties (e.g., compare ref. 8 with 15). Thus, renaturation rates and accompanying density shifts in CsCl seem to be insufficient criteria for determining the subcellular location of a particular DNA species in plants (24). All-in-all, the various examples cited in this discussion, together with our own results, suggest that a re-examination of the function(s) and subcellular location(s) of the satellite DNAs in plants might be fruitfully pursued.

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1. Degani, Y. & Atsmon, D. (1970) *Nature* **228**, 554–555.
2. Degani, Y. & Atsmon, D. (1970) *Exp. Cell Res.* **61**, 226–229.
3. Kadouri, A. & Atsmon, D. (1974) in *Mechanisms of Regulation of Plant Growth*, eds. Bieleski, R. L., Ferguson, A. R. & Cresswell, M. M. (Roy. Soc. New Zealand, Wellington), *Bull.* **12**, 339–343.
4. Matsuda, K. & Siegel, A. (1967) *Proc. Nat. Acad. Sci. USA* **58**, 673–680.
5. Beridze, T. G., Odintsova, M. S. & Sisakyan, N. M. (1967) *Mol. Biol.* **1**, 122–133 (English transl.).
6. Quétier, F., Guillé, E. & Vedel, F. (1968) *C.R.H. Acad. Sci. Ser. D.* **266**, 735–738.
7. Ingle, J., Pearson, G. G. & Sinclair, J. (1973) *Nature New Biol.* **242**, 193–197.
8. Bendich, A. J. & Anderson, R. S. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 1511–1515
9. Walker, P. M. P. (1971) *Prog. Biophys. Mol. Biol.* **23**, 145–190.
10. Thornberg, W. & Siegel, A. (1973) *Biochemistry* **12**, 2759–2765.
11. Matsuda, K., Siegel, A. & Lightfoot, D. (1970) *Plant Physiol.* **46**, 6–12.
12. Pearson, G. G., Timmis, J. N. & Ingle, J. (1974) *Chromosoma* **45**, 281–294.
13. Tewari, K. K. & Wildman, S. G. (1966) *Science* **153**, 1269–1271.
14. Edelman, M. (1974) in *Methods in Enzymology*, eds. Jakoby, W. B., & Wilchek, M. (Academic Press, New York), Vol. 34B, pp. 499–502.
15. Wells, R. & Birnstiel, M. (1969) *Biochem. J.* **112**, 777–786.
16. Gadaleta, M. N., Barletta, A., Caldarazzo, M., DeLeo, T. & Saccone, C. (1972) *Eur. J. Biochem.* **30**, 376–381.
17. Tautvydas, K. J. (1971) *Plant Physiol.* **47**, 499–503.
18. Marmur, J. (1961) *J. Mol. Biol.* **3**, 208–218.
19. Mandel, M., Schildkraut, C. L. & Marmur, J. (1968) in *Methods in Enzymology*, eds. Grossman, L. & Moldave, K. (Academic Press, New York), Vol. 12B, pp. 184–195.
20. Calvayrac, R., Butow, R. A. & Lefort-Tran, M. (1972) *Exp. Cell Res.* **71**, 422–432.
21. Osafune, T., Mihara, S., Hase, E. & Ohkuro, I. (1972) *Plant Cell Physiol.* **13**, 211–227.
22. Green, B., Heilporn, V., Limbosch, S., Boloukhere, M. & Brachet, J. (1967) *Proc. Nat. Acad. Sci. USA* **58**, 1351–1358.
23. Edelman, M., Epstein, H. T. & Schiff, J. A. (1966) *J. Mol. Biol.* **17**, 463–469.
24. Herrmann, R. G., Bohnert, H.-J., Kowallik, K. V. & Schmitt, J. M. (1975) *Biochim. Biophys. Acta* **378**, 305–317.
25. Atsmon, D. (1972) in *Plant Growth Substances, 1970*, ed. Carr, D. J. (Springer Verlag, New York), pp. 222–227.
26. Atsmon, D., Lang, A. & Light, E. N. (1968) *Plant Physiol.* **43**, 806–810.
27. *Soc. Exp. Biol. Symp.* **24** (1970) "Control of Organelle Development," ed. Miller, P. L. (Cambridge Univ. Press, New York).
28. Smillie, R. M. & Scott, N. S. (1969) in *Progress in Molecular and Subcellular Biology*, eds. Hahn, F. E., Puck, T. T., Springer, G. F., Szybalski, W. & Wallenfels, K. (Springer Verlag, New York), Vol. I, pp. 136–202.
29. Woodcock, C. L. F. & Bogorad, L. (1971) in *Structure and Function of Chloroplasts*, ed. Gibbs, M. (Springer Verlag, New York), pp. 89–128.
30. Wells, R. & Ingle, J. (1970) *Plant Physiol.* **46**, 178–179.
31. Kirk, J. T. O. (1971) in *Autonomy and Biogenesis of Mitochondria and Chloroplasts*, eds. Boardman, N. K., Linnane, A. W. & Smillie, R. M. (North Holland, Amsterdam, Neth.), pp. 267–276.
32. Tewari, K. K. & Wildman, S. G. (1970) *Soc. Exp. Biol. Symp.* **24**, 147–179.
33. Machie, R. & Waygood, E. R. (1969) *FEBS Lett.* **3**, 89–92.
34. Kessler, B. (1973) in *The Biochemistry of Gene Expression in Higher Organisms*, eds. Pollard, J. K. & Lee, J. W. (Australia and New Zealand Book Co., Artarmon), pp. 333–356.
35. Sparrow, A. H., Price, H. J. & Underbrink, A. G. (1971) *Brookhaven Symp. Biol.* **23**, 451–494.
36. Bennet, M. D. (1972) *Proc. Roy. Soc. Ser. B* **181**, 109–135.