HORMONE EFFECT ON THE HALF-LIFE OF THE 42 S pre-rRNA OF CULTURED SYCAMORE CELLS

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1. Introduction

Ribosomal RNAs are formed through a series of modifications of the initial transcript of ribosomal cistrons. This includes successive cleavages of the transcript, addition of methyl groups and conversion of uridine to pseudouridine [1]. The physiological significance of these post-transcriptional events is still unknown. However, it has been proposed that they are possible control points for the production of ribosomes [1,2] and may be key factors in the regulation of cell metabolism.

In recent years, considerable attention has been devoted to the various factors which control the growth of plant cells. Several systems are now known in which transcriptional and translational controls are achieved by hormones or growth regulators [3], but little is known about possible hormonal effects on the various steps of the formation of ribosomes.

We have reported on the nucleolytic reactions leading from the transcript of ribosomal cistrons of cultured sycamore cells (2.9×10^6 daltons) to the mature cytoplasmic rRNAs, via a limited number of intermediates (2.3×10^6, 1.4×10^6 and 0.9×10^6 daltons) [4–6]. The base and ribose methylation of the mature ribosomal sequences had been also investigated [7,8]. The present paper reports on the effect of 2,4-dichlorophenoxyacetic acid (2,4-D) on the processing of the 2.9×10^6 pre-rRNA.

2. Materials and methods

Batch-propagated sycamore (*Acer pseudoplatanus* L.) cells were normally grown as in [4] in the presence of 2,4-dichlorophenoxyacetic acid (2,4-D, 1 mg/l). Stock cultures were maintained in logarithmic growth phase by successive transfers of aliquots (~100 ml) every 10–12 days to standard fresh medium (~500 ml). Cells deprived of exogenous auxin were obtained according to the following procedure. The liquid medium of a 10 day culture was eliminated by filtering on a 50 μm diameter pore filter. After several rinses of the cells with fresh medium deprived of 2,4-D, the filter was cut up and cells were resuspended in minus-2,4-D medium at $\sim 4 \times 10^4$ cells/ml. In some instances, the medium of a stock culture was entirely renewed by the same procedure. Growth of cultures was studied by measuring both cell density and nucleic acid content. Cell densities were estimated according to [9]. Nucleic acids were extracted by the method in [10]. DNA was determined by the assay in [11]. RNA was estimated by measuring total nucleic acid [12] and subtracting DNA.

For isotope uptake and incorporation measurements, $\sim 10^7$ cells were incubated with 100 μCi of either [^{3}H]uridine (10 Ci/mM) or [^{3}H]leucine (125 mCi/mM) in 25 ml culture medium for 15 min. Cells were then rapidly cooled, rinsed with a cold saccharose solution (20 g/l) and disrupted with a MSE sonifier in 20 mM Tris–HCl buffer (pH 7.2), 10 mM NaCl, 10 mM EDTA. For measurement of the incorporation of [^{3}H]leucine into proteins, an aliquot of the corresponding homogenate was made 5% trichloroacetic acid. The precipitate was collected by centrifugation, washed with 5% trichloroacetic acid and resuspended in Tris–HCl buffer. Total RNAs were prepared by the phenol-extraction procedure [4]. The total radio-

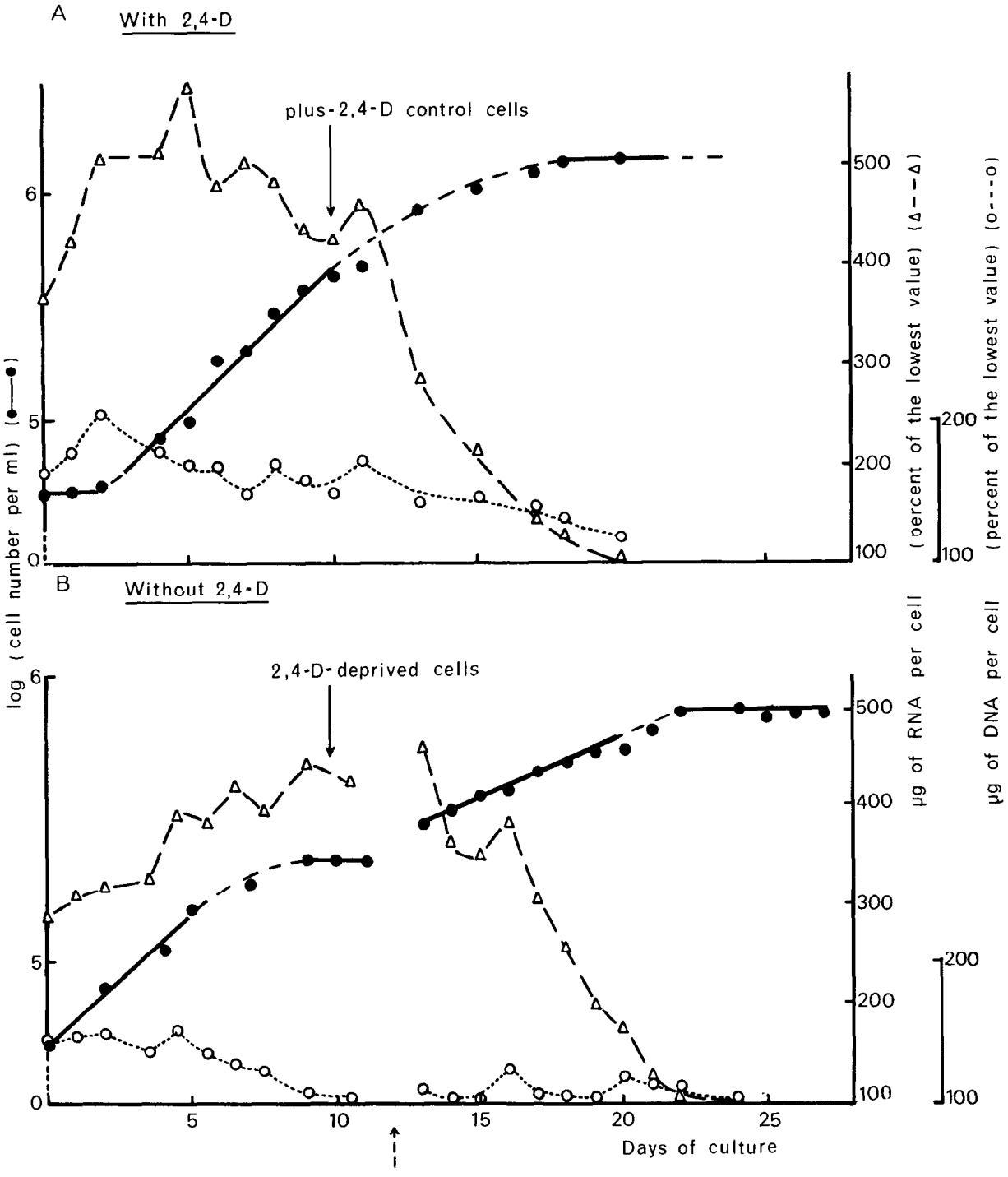
A
With 2,4-D
plus-2,4-D control cells
B
Without 2,4-D
2,4-D-deprived cells
log (cell number per ml) (●—●)
(percent of the lowest value) (Δ--Δ)
(percent of the lowest value) (o---o)
µg of RNA per cell
µg of DNA per cell
Days of culture

Fig.1

activity of the homogenates, RNA and protein solutions were estimated by liquid scintillation counting [4]. Correction for variations in the efficiency of counting was made.

All methods for the study of nuclear RNA were as in [4–6].

3. Results and discussion

3.1. *Subculture of sycamore cells into a minus-2,4-D medium*

Growth of sycamore cell suspensions depends on the presence of 2,4-D in the culture medium [13]. Figure 1A shows the typical S-shaped curve obtained when cells from an exponentially growing culture (stock culture, see section 2) were washed and inoculated in a totally renewed medium provided with 2,4-D (1 mg/l). It has already been shown that the initial lag phase is due to the removal from the culture medium of endogenous growth substances [14] and that nitrogen is the limiting factor responsible for the stationary phase [15]. When cells from a stock culture were subcultured into a totally renewed medium, but deprived of 2,4-D (fig.1B), the growth stopped after only 1–2 doublings of the cell population (first stationary phase) compared with the 4–5 doublings in 2,4-D-supplied cultures (fig.1A). However, upon readdition of 2,4-D (1 mg/l) to the culture medium cell divisions started again producing an additional 4-fold increase in cell number. It is noteworthy that, except for the interruption corresponding to the first stationary phase, the whole trend of DNA and RNA content was similar to that observed in fig.1A. Obviously, 2,4-D is the limiting factor responsible for the first stationary phase. Hormone effects on the processing of the larger pre-rRNA were then investigated in subcultured cells arrested in the first stationary phase (fig.1B) (2,4-D-deprived cells). The auxin was usually re-added to the culture medium 5–6 days after growth was stopped. Cells from a day 10 stock culture (plus 2,4-D control cells) were taken as reference for estimation of the extent of hormone action.

3.2. *Viability of the 2,4-D-deprived cells*

That 2,4-D-deprived cells were living was indicated by the rapid recovery of cell division after re-addition of the auxin to the culture medium. This was further tested by measuring the transcriptional and translational activities of the cells (tables 1, 2). As reported in table 1, 2,4-D-deprived cells incorporated significant amounts of uridine and leucine into nucleic acids and proteins. After 15–30 min hormone treatment RNA and protein syntheses increased considerably and were close to those observed in stock cells (plus-2,4-D control cells) (see legend of table 1). This early increase of incorporation was actinomycin D-sensitive. No further increase was observed for longer treatments of the cells with 2,4-D. This is in agreement with the numerous reports on hormone effects on transcription and translation [16].

Since both 2,4-D and actinomycin-D were found to strongly affect the uptake of the radioactive precursor by the cells, all incorporation values were expressed as the ratio of the amount of isotope incorporated into macromolecules to that of isotope effectively present in the cells. This implied that the level of incorporation of a radioactive precusor into the corresponding macromolecules is linearly related to its uptake by the cells. As illustrated in table 2, this was roughly verified. From the data reported in fig.1 and tables 1, 2 it may be concluded that the 2,4-D-deprived cells respond rapidly to the hormone and may be a convenient tool for the study of 2,4-D effects at the molecular level.

Fig.1. Growth of sycamore cells in the presence or absence of 2,4-D in the culture medium. According to procedure stated in section 2, dividing cells from a stock culture were transferred to a totally new medium provided (A) or not (B) with 2,4-D (1 mg/l). Aliquots of the cultures were withdrawn and cell densities and nucleic acids estimated as indicated in section 2. The broken arrow (B) shows the moment of the re-addition of 2,4-D (1 mg/l) to the culture medium. DNA and RNA contents are expressed as percent of the minimum value reached at the end of the growth cycle (about 12.5×10^{-6} μg/cell and 10×10^{-6} μg/cell, respectively, for DNA and RNA). These values were approximately the same for both the cultures.

Table 1
Rate of incorporation of [^{3}H]uridine and [^{3}H]leucine into RNA and proteins in 2,4-D-deprived cells. Time course for the effect of 2,4-D re-addition to the culture medium

Duration of treatment of the cells with 2,4-D	[^{3}H]leucine[a] (pmol × min^{-1} × mg protein^{-1})	[^{3}H]uridine[b] (pmol × min^{-1} × mg RNA^{-1})
Without preincubation		
0 h	3.9	28
0 25–0.5 h	5 3–5.9	62–70
6–8 h	6.2–7.0	56–59
After 3 h preincubation with 50 μg/ml actinomycin D		
0 h	–	5.6
0.25–0.5 h	3.7–4 1	6.7
6–8 h	5.1–5.5	7 6

Sycamore cells subcultured into a minus-2,4-D medium were treated as indicated 5–6 days after growth was stopped (see fig.1B) and then tested for protein and RNA synthesis as specified in section 2. Results are expressed as pmol incorporated isotope/1000 pmol isotope present in the cell/min incubation and [a]mg proteins or [b]RNA. For comparison, the measured values for cells from a day 10 stock culture (plus-2,4-D control cells) were 6.5 and 90 for leucine and uridine, respectively

Table 2
Uptake and incorporation of [^{3}H]uridine by actinomycin D-treated sycamore cells incubated in the presence of various amounts of the labelled precursor

Amount of [^{3}H]uridine in the culture medium	[^{3}H]uridine[a] (pmol × min^{-1} × mg RNA^{-1})		Ratio 2:1 × 10^3
	Present in the cell[1]	Incorporated into RNA[2]	
100 μCi	84	1.2	14.3
200 μCi	221	2.6	11.8
400 μCi	474	6.1	12.9

[a] The control values for cells grown without actinomycin D and incubated with 100 μCi [^{3}H]uridine were· uptake 358, incorporation 5.1 and incorporation/uptake ratio 14.2 × 10^{-3}. These results cannot be compared to those reported in table 1 since different labelling conditions were used

Cells from a day 10 stock culture were incubated with 5 μg/ml actinomycin D for 12 h. Aliquots (~10^8 cells in 65 ml culture medium) received [^{3}H]uridine (10 Ci/mM) for 15 min, as indicated. Uptake and incorporation of labelled uridine were estimated as reported in section 2. Result are expressed as pmol precursor/min incubation and mg RNA

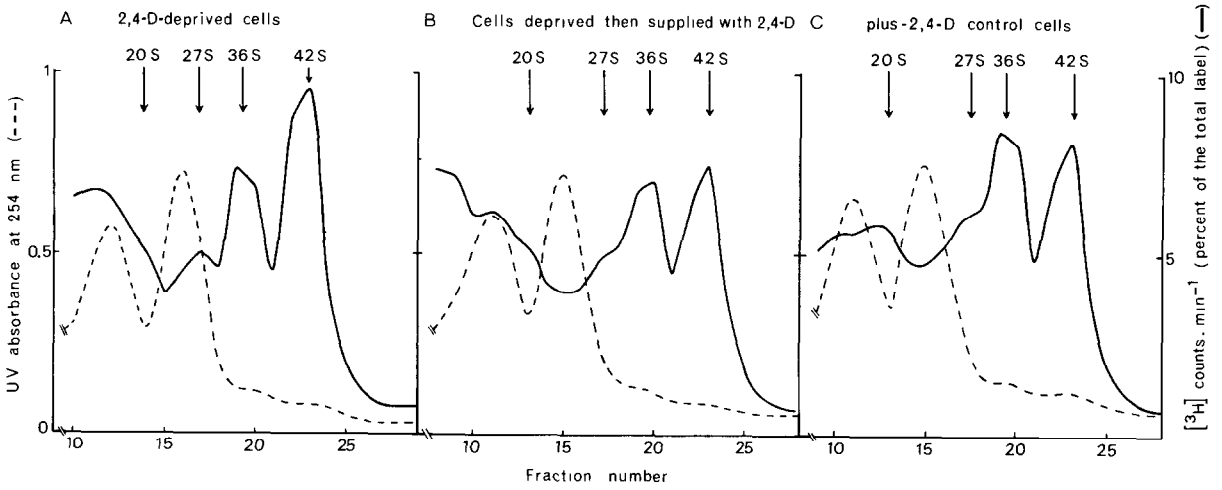

Fig.2. Sucrose gradient analysis of *Me*-^{3}H-labelled nuclear RNA from sycamore cells supplied or not with 2,4-D. 2,4-D-deprived cells (A) and 2,4-D-deprived cells treated with the auxin for 2 h (B) received [*Me*-^{3}H]methionine for 15 min as in [4]. For comparison plus-2,4-D control cells were treated in the same way (C). Nuclear RNAs were extracted with the phenol-extraction procedure [4] and sedimented on 10–30% sucrose gradients with a Spinco SW 25-1 rotor at 18°C and 22 500 rev./min for 15 h. Only the relative distribution of the label from the 10–60 S regions of the gradients is reported.

3.3. *Effects of 2,4-D on the maturation of pre-rRNA*

As reported in [4,6], methyl-labelled methionine is the more specific radioactive precursor available for investigating the maturation pathway of rRNA. It was used in the following experiments. Figure 2 shows the sucrose gradient analyses of *Me*-^{3}H-labelled nuclear RNAs isolated from 2,4-D-deprived cells (fig.2A), 2,4-D-deprived cells supplemented with auxin for 2 h (fig.2B) and from plus-2,4-D control cells (fig.2C). The 4 classes of pre-rRNA (42 S, 36 S, 27 S and 20 S) were present in all RNA samples, indicating that 2,4-D did not alter the number of rRNA intermediates. However, the radioactivity profiles reflected some differences in the relative amounts of the pre-rRNA species. The proportion of the 42 S molecule was greater in 2,4-D-deprived cells and after a 2 h hormone treatment the relative distribution of the label among the 42 S and 36 S RNA became similar to that observed in plus-2,4-D control cells. These data strongly suggest that 2,4-D acts on the turn-over of the largest pre-rRNA. This was verified by performing comparative pulse-chase experiments on 2,4-D-deprived cells and plus-2,4-D control cells (not illustrated). The procedure was as in [6]. No differences were observed in the maturation pathway of pre-RNA in the presence or the absence of auxin. However, from a semi-logarithmic plot of the label incorporated into the 42 S pre-rRNA versus the time-chase (fig.3) it might be seen that

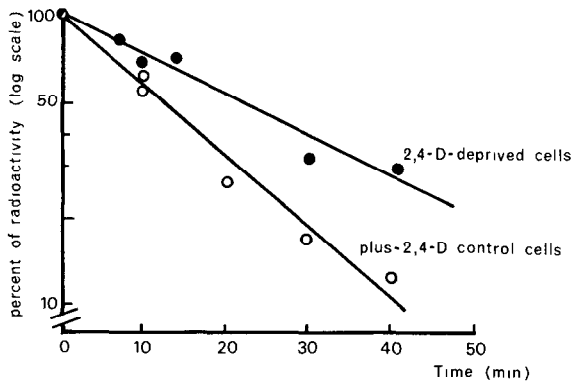

Fig.3. Effect of 2,4-D on the half-life of the initial transcript of ribosomal cistrons in sycamore cells. 2,4-D-deprived cells and plus-2,4-D control cells were pulse-labelled with [*Me*-^{3}H]-methionine and then chased with an excess of cold methionine as in [4,6]. Identical aliquots were withdrawn at various period of chase. Nuclear RNAs were prepared and sedimented on sucrose gradients as in fig.2. The label of the 42 S pre-rRNA was estimated and plotted versus the duration of the chase period.

2,4-D significantly alters the conversion of the 42 S rRNA into the 36 S rRNA. In the absence of auxin the half-life of the initial transcript is approximately double that found in the presence of hormone.

Obviously, the overall production of rRNA depends upon both the rate of transcription of the ribosomal genes and the efficiency of processing of their transcript. While the former point is now well documented, no conclusive data were available for the latter, either in animal or plant cells. As regard to higher plants, a general model of the mode of action of auxin on gene expression has been proposed [17]. Here, we report evidence of an auxin effect on the rate of conversion of the primary transcript of ribosomal cistrons in sycamore cells. Whether the synthesis of the nucleolytic enzymes involved in the processing is stimulated by 2,4-D or their activity modulated via some 2,4-D-induced modification of the 3-D structure of the transcript is an interesting question. In the latter hypothesis, possible changes in the methylation pattern of the pre-RNAs, would have to be considered.

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