Transcription program of the chloroplast genome of *Euglena gracilis* during chloroplast development

(restriction endonuclease fragments/nick translation/RNA-DNA hybridization kinetics/transcription mapping)

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ABSTRACT RNA transcription in *Euglena gracilis* chloroplasts has been characterized by hybridization of RNA from cells at different stages of chloroplast development to ³H-labeled chloroplast DNA restriction endonuclease fragments. Chloroplast DNA was digested into five fragments of 53,000, 35,000, 25,000, 10,000, and 6900 base pairs with *Pst* I. The 53,000-base-pair DNA was further cleaved by *Bam*HI digestion. Eight different DNA fragments, of known restriction nuclease map location and accounting for the entire genome, were labeled *in vitro* by means of the nick translation reaction of DNA polymerase I with [³H]dTTP as a substrate. RNA was isolated from dark-adapted *Euglena* cells, and from cells at an early (4 hr) and a late (72 hr) stage of light-induced chloroplast development. The RNAs were hybridized in solution to each ³H-labeled chloroplast DNA fragment. From the extents and kinetics of the reactions, a temporal program for RNA transcription from defined regions of the chloroplast genome could be described. Different classes of transcription units are present, including RNAs (i) continuously present throughout development, (ii) induced at the onset of development, (iii) repressed early in development, and (iv) induced late in development.

When *Euglena* is grown in the dark on an oxidizable carbon source the cells lose the photosynthetic pigments and lamellae of the chloroplasts. The chloroplasts also lose the components of photosynthetic electron transport and the ability to fix CO₂. In the dark, chloroplasts are replaced by proplastids, an organelle containing chloroplast DNA surrounded by a double membrane (1, 2). When dark-adapted cells are shifted to the light, chloroplast development begins whether the cells are dividing or not. Development is a temporal process, marked by the acquisition of properties of mature chloroplasts at specific intervals after the onset of light-induced development. The process is complete after 72 hr, with each cell having a full complement of mature chloroplasts (1, 2).

We have been studying the changes in RNA transcription in the chloroplasts during light-induced development. *Euglena* chloroplast DNA, which has a genome size of 130,000–140,000 base pairs (130–140 kbp) (3, 4), is extensively transcribed at all developmental stages (5, 6). However, the extent of transcription varies significantly with both the developmental stage and the culture conditions. In exponentially growing cells on a phototrophic medium, there is an initial decrease in the fraction of the genome transcribed during the first 4 hr of growth in the light. Subsequently an induction of RNA synthesis occurs, reaching a maximum value 72 hr after the onset of development. At this time 23–26% of the DNA is transcribed, yielding RNA of 60–65 kilobase (kb) complexity (5, 7).

Since these results were reported, a detailed restriction endonuclease map of the *Euglena* chloroplast genome has been published (4, 8). With the map as reference, it has been possible to locate regions of the genome containing developmentally regulated transcription units. Specific restriction fragments

from chloroplast DNA were purified, radioactively labeled to high specific activity *in vitro*, and utilized as hybridization probes for specific regions of the chloroplast genome. The restriction fragments used in this study were of known position on the chloroplast genome, and in aggregate represent the entire DNA sequence content. By using these hybridization probes, transcription was quantitatively studied at three stages: before induction of chloroplast development in dark-adapted cells, early during chloroplast development, and at a late stage of chloroplast development. From the results it can be concluded that the chloroplast genome contains several different types of transcription units, which can be characterized by their pattern of regulation.

METHODS

Preparation of RNA. The preparation of dark-adapted cultures of *Euglena gracilis* Klebs (strain Z Pringsheim cells), the protocol for obtaining greening cultures in a photoautotrophic medium, the procedure for harvesting cells at various stages of chloroplast development, and the method of cell RNA isolation have been described (5). Total cellular *Euglena* RNA was isolated from cells harvested at 0, 4, and 72 hr of chloroplast development.

Preparation of Hybridization Probes. The preparation of ³H-labeled chloroplast DNA restriction endonuclease fragments has been described (7). Hybridization of total cell RNA to ³H-labeled restriction fragments in solution, detection of hybrids by using an S1 nuclease criterion, and mathematical treatment of the data have been described (5, 7).

RESULTS

Fractionation of the Chloroplast Genome. To quantitatively study RNA transcription from isolated regions of the *Euglena* chloroplast genome, chloroplast DNA was fragmented by the action of restriction endonucleases. For the studies to be described, the five restriction fragments produced by the enzyme *Pst* I (*Pst*-A–E), two of the restriction fragments produced by the combination of enzymes *Bam*HI and *Pst* I (*BP*-3 and *BP*-4), and the fragment *Eco*-H produced by the enzyme *Eco*RI were utilized. These fragments are positioned relative to one another as previously illustrated (7). They represent a complete set of isolated regions of the chloroplast genome. The rRNA genes are arranged as a triple tandem repeat in approximately the center of the 53 kbp *Pst*-A region. Radioactive labeling of DNA *in vitro* to a specific activity of 1.1–3.6 μCi/μg of DNA (1 Ci = 3.7 × 10¹⁰ becquerels) was achieved by the action of DNA polymerase I with [³H]dTTP as a substrate.

Abbreviations: kbp, 1000 (kilo-) base pairs; kb, kilobase.

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Table 1. Analysis of the hybridization of developing *Euglena* RNA to the chloroplast DNA *Pst*-A region

Probe	RNA driver, hr	Transition	$R_{0t_{1/2}}$ obs., M·sec	Complexity,* kb	$R_{0t_{1/2}}$ calc., [†] M·sec	Total RNA, [‡] %
<i>Pst</i> -A	0	1	0.038	14.3	0.0027	7.1
		2	14	12.0	0.0068	0.048
		1 + 2	—	26.3 ± 0.64 [§]	—	—
	4	1	0.063	14.8	0.0028	4.4
		2	20	7.5	0.0042	0.021
		1 + 2	—	22.3 ± 2.6 [§]	—	—
	72	1	0.015	13.8	0.0026	17
		2	5.4	16.0	0.0090	0.17
		1 + 2	—	29.8 ± 0.74 [§]	—	—

* Calculated as the fraction of the hybridization probe hybridized at the completion of the reaction times the single-stranded size of the probe. For *Pst*-A this value is corrected for contamination with *Pst*-B and for the base compositional heterogeneity of *Pst*-A as described in ref. 7.

[†] Calculated from: $R_{0t_{1/2}}$ standard/complexity standard = $R_{0t_{1/2}}$ calc. unknown/complexity unknown. The standard used was a $R_{0t_{1/2}}$ = 2.7×10^{-3} M·sec for a complexity = 4.8 kb as determined for *Euglena* chloroplast rRNA (9). The complexity of the *Pst*-A high-abundance transcripts was divided by 3 because these are the three copies of the chloroplast rRNA genes in *Pst*-A (see text).

[‡] Calculated from: % total RNA = $100 \times R_{0t_{1/2}}$ calc./ $R_{0t_{1/2}}$ obs.

[§] The total complexity values are the mean ± SEM of 10, 6, and 8 determinations for 0-, 4-, and 72-hr RNA, respectively. The 0- and 4-hr saturation values are significantly different, $P < 0.20$. The 0- and 72-hr values are significantly different, $P < 0.05$.

Hybridization of Chloroplast [³H]DNA Restriction Fragments. Chloroplast [³H]DNA restriction fragments were used as hybridization probes in RNA-driven hybridization reactions to quantitate the transcription of specific regions of the chloroplast genome. Each hybridization probe was hybridized to a vast excess of three different RNA samples and in some cases combinations of RNA samples. The "driver" RNAs were isolated from *Euglena* cells harvested after 0, 4, and 72 hr of chloroplast development. The kinetics of the hybridization reactions were followed by measuring the fraction of the appropriate [³H]DNA hybridization probe that was insensitive to the single strand-specific S1 nuclease. Both the initial RNA concentration (R_0 , in moles of nucleotide per liter) and the reaction time (t) were varied. A correction was made for the competing DNA-DNA renaturation reaction by carrying out a comparable reaction without the driver RNA and subtracting this value to yield the RNA-DNA fraction. The DNA concentration was kept low to minimize DNA-DNA renaturation.

***Pst*-A Region.** The *Pst*-A fragment of the *Euglena* chloroplast genome is 53 kbp. When [³H]*Pst*-A is hybridized to a large excess of total cellular RNA from cells at different stages of chloroplast development, the reactions proceed as illustrated in Fig. 1. The fraction of the hybridization probe in the form of RNA-DNA hybrids at the completion of the reaction varies with the stage of development. Various amounts of the *Pst*-A region are transcribed into detectable amounts of RNA during chloroplast development. At each stage of development, the hybridization kinetics are consistent with at least two cellular RNA classes hybridizing with *Pst*-A. The data can be fit as a biphasic reaction at the three stages of development tested. The biphasic reaction is indicative of two classes of RNA, high abundance and low abundance. Quantitative analyses of these reaction kinetics in terms of two-component RNA populations are listed in Table 1.

The chloroplast rRNA genes have previously been mapped in the *Pst*-A region of the chloroplast genome (4, 10, 11). There are three sets of 16S and 23S rRNA genes that total approximately 14.4 kbp in size. It can be seen from the analyses listed in Table 1 that the chemical complexity of the high-abundance *Pst*-A RNA transcripts (14–15 kb, depending on the stage of development) is in agreement with its assignment as rRNA. The abundance of these transcripts is also in agreement with the

abundances previously determined by using a hybridization probe specific for chloroplast rRNA (9). It may therefore be concluded that the cellular RNA that hybridizes with *Pst*-A at low R_0t values is chloroplast rRNA.

In contrast to the high-abundance RNA, which accounts for a transcribed region of constant size, the low-abundance *Pst*-A transcripts vary in complexity during development. There is a 4.5-kb decrease in the complexity of the transcribed region during the first 4 hr of chloroplast development. These transcripts do not disappear by dilution, because the generation time of these developing cells is 24 hr. The RNA transcripts from this 4.5-kb region must therefore be degraded after the onset of development. The size of the transcribed regions of *Pst*-A at 72 hr is 8.5 kb greater than at 4 hr, and 4.0 kb greater than at 0 hr of development. This is strong evidence that new RNA transcripts induced at the later stages of chloroplast development are not present in dark-grown cells or cells at early stages of development. The level to which the *Pst*-A low-abundance transcripts accumulate varies slightly throughout chloroplast development, ranging from 0.021% to 0.17% of the total cell RNA (Table 1).

The non-rRNA transcription of the *Pst*-A region of the chloroplast genome can be studied independently of the rapidly

FIG. 1. Hybridization of RNA from different stages of *Euglena* chloroplast development to [³H]*Pst*-A. Total cell RNA was extracted from cells prior to the onset of light-induced development (0 hr, ○), at an early development stage (4 hr, △), and late in development (72 hr, □). RNA was hybridized in vast excess of [³H]*Pst*-A.

FIG. 2. Hybridization of RNA from different stages of chloroplast development to $[^3\text{H}]BP-3$ (Upper) and $[^3\text{H}]BP-4$ (Lower). RNA from cells at 0 hr (O), 4 hr (Δ), and 72 hr ($\square$) of light-induced development was used. Values reported are duplicate measurements at each R_0t value. The continuous lines in the figure are theoretical curves for a single-component, pseudo-first-order reaction with the $R_0t_{1/2}$ and complexity values reported in Table 2.

hybridizing rRNA component by the use of hybridization probes made from the chloroplast DNA restriction fragments *BP-3* and *BP-4*, which are subfragments of *Pst-A* (7). The two fragments total 93% of the non-rDNA regions of the *Pst-A* fragment. *BP-3* contains no rRNA-coding sequences. The 14-kbp *BP-4* (or *Bam-C*; ref. 4) contains <0.1 kbp of a 5S rRNA gene. The kinetics of the hybridization of RNA from cells at various stages of chloroplast development with $[^3\text{H}]BP-3$ and $[^3\text{H}]BP-4$ are illustrated in Fig. 2. For quantitative analysis of these reactions, we have treated these data as single-component pseudo-first-order hybridizations. This treatment should be considered as only an approximation. These results are shown in Table 2. The sizes of the *BP-3* and *BP-4* transcribed regions are in agreement with the sizes of the *Pst-A* regions that yield low-abundance transcripts. The non-rRNA *Pst-A* transcripts have complexities of 12.0, 7.5, and 16.0 kb at 0, 4, and 72 hr of development, respectively (Table 1), while the sum of the complexities of the *BP-3* and *BP-4* transcripts are 12.5, 8.5, and 13.3 kb (Table 2), respectively, for the same time periods. The

Table 2. Analysis of the hybridization of developing *Euglena* RNA to the chloroplast DNA *BP-3* and *BP-4* regions*

Probe	RNA driver, hr	$R_0t_{1/2}$ obs., M·sec	Complexity, kb	$R_0t_{1/2}$ calc., M·sec	Total RNA, %
<i>BP-3</i>	0	8.1	6.7	0.0038	0.047
	4	12.8	3.8	0.021	0.016
	72	6.5	7.1	0.0040	0.062
	0 + 72	—	6.7	—	—
<i>BP-4</i>	0	7.2	5.8	0.0033	0.046
	4	3.7	4.7	0.0026	0.070
	72	7.4	6.2	0.0035	0.047

* See footnotes to Table 1 for calculations. The RNA population is approximated as a single kinetic component in each case of the above analyses. The same approximation was made in the analyses reported in Tables 4 and 5.

FIG. 3. Hybridization of $[^3\text{H}]Pst-B$ with RNA from *Euglena* cells at 0 hr (O), 4 hr (Δ), and 72 hr ($\square$) of chloroplast development. The values reported are the averages of two determinations. The continuous lines are theoretical curves for two-component hybridization reactions, based on the $R_0t_{1/2}$ and complexity values reported in Table 3.

slight difference in these two experiments may be a result of two non-rRNA-coding regions of *Pst-A* totaling 2.6 kbp, which are not part of either *BP-3* or *BP-4*. The RNA transcripts from a 3-kb region of *BP-3* disappear from the cell in the first 4 hr of development while the transcripts from a 1-kb region of *BP-4* behave similarly. At the completion of development, an additional 3.3 kb and 1.5 kb of *BP-3* and *BP-4*, respectively, are being transcribed over the 4-hr level. When 0-hr and 72-hr RNA are mixed and hybridized to *BP-3*, the saturation value is the same as that obtained with 72-hr RNA alone (Table 2). From these results it can be concluded that the accumulation of some low-abundance *Pst-A* transcripts is inhibited during the first 4 hr of development, but these RNAs reappear later during development. In addition, the expression of previously "silent" regions in *Pst-A* occurs after 4 hr of development.

***Pst-B* Region.** The *Pst-B* fragment of the chloroplast genome is 35 kbp. The hybridization kinetics of $[^3\text{H}]Pst-B$ to RNA from cells at different stages of chloroplast development are illustrated in Fig. 3. The saturation level for the hybridization of the *Pst-B* region varies throughout development. There is a significant decrease in the saturation level during the first 4 hr of chloroplast development followed by a large increase between 4 and 72 hr. We have treated the hybridization kinetics as a two-component pseudo-first-order reaction. This is an approximation at best for these data, because the high-abundance RNA class in 0-hr RNA is heterogeneous and actually represents the sum of several RNA abundance classes. There is additional uncertainty in studying the high-abundance *Pst-B* component because the hybridization reaction involves only a small fraction of the hybridization probe. The analyses of these reactions are listed in Table 3.

FIG. 4. Hybridization of $[^3\text{H}]Pst-C$ with RNA from *Euglena* cells at 0 hr ($\bullet$), 4 hr ($\blacktriangle$), and 72 hr ($\blacksquare$) of chloroplast development. The values reported are the averages of two determinations.

Table 3. Analysis of the hybridization of developing *Euglena* RNA to the chloroplast DNA *Pst*-B region*

Probe	RNA driver, hr	Transition	R _{0t} _{1/2} obs., M-sec	Complexity, kb	R _{0t} _{1/2} calc., M-sec	Total RNA, %
<i>Pst</i> -B	0	1	0.011	4.4	0.0025	(23) [†]
		2	10	10.3	0.0058	0.058
		1 + 2	—	24.7 ± 0.28 [‡]	—	—
	4	1	0.098	2.9	0.0016	(1.6) [†]
		2	11	8.9	0.0050	0.045
		1 + 2	—	11.8 ± 0.35 [‡]	—	—
	72	1	0.012	5.8	0.0037	(28) [†]
		2	12	11.6	0.0065	0.054
		1 + 2	—	17.4 ± 0.84 [‡]	—	—
	0 + 4	1 + 2	—	15.4	—	—
	0 + 72	1 + 2	—	16.1	—	—

* See footnotes to Table 1 for calculations. For *Pst*-B this value is corrected for contamination with *Pst*-A as described in ref. 7.

[†] There is considerable uncertainty in the analysis of the high-abundance transcripts in *Pst*-B. See the text for explanation.

[‡] The total complexity values are the mean ± SEM of 10, 10, and 8 determinations for the 0-, 4-, and 72-hr RNA, respectively. The 0- and 4-hr values are significantly different, $P < 0.01$. The 0- and 72-hr values are significantly different, $P < 0.01$.

Both the low-abundance and high-abundance *Pst*-B RNA classes decrease in complexity during the first 4 hours of chloroplast development and then increase significantly at 72 hr of development. The high-abundance *Pst*-B RNA transcripts represent a large fraction of the total cellular RNA. The low-abundance *Pst*-B transcripts, on the other hand, remain approximately constant at 0.05% of the cellular RNA. When 0-hr and 4-hr RNA are mixed and hybridized to [³H]*Pst*-B, the saturation value is not significantly different than with 0-hr RNA alone (Table 3). Similarly, when 0-hr RNA is mixed with 72-hr RNA and hybridized to [³H]*Pst*-B, the saturation value is the same as with 72-hr RNA alone. Therefore, the 4-hr transcripts are a subset of the 0-hr transcript population and the 0- and 4-hr RNA transcripts are subsets of the 72-hr RNA population. From these results it can be concluded that the accumulation of some of the *Pst*-B transcripts is inhibited during the first 4 hr of development. This is followed by a reactivation of these transcripts, and the expression of previously "silent" *Pst*-B genes. Both low- and high-abundance *Pst*-B transcripts exhibit this pattern of expression.

***Pst*-C Region.** The *Pst*-C fragment of the chloroplast genome is 25 kbp. The hybridization kinetics of [³H]*Pst*-C to RNA from cells at different stages of chloroplast development are illustrated in Fig. 4. The saturation level of the *Pst*-C region significantly increases from 0 to 4 hr of chloroplast development, followed by a further increase from 4 to 72 hr. The quantitative analyses of these hybridization reactions are listed in Table 4.

Table 4. Analysis of the hybridization of developing *Euglena* RNA to the chloroplast DNA *Pst*-C region*

Probe	RNA driver, hr	R _{0t} _{1/2} obs., M-sec	Complexity, kb	R _{0t} _{1/2} calc., M-sec	Total RNA, %
<i>Pst</i> -C	0	4.9	8.8 ± 0.20 [†]	0.0050	0.10
	4	7.0	11.1 ± 0.50 [†]	0.0062	0.088
	72	9.8	15.0 ± 0.35 [†]	0.0084	0.086
	4 + 72	—	14.8	—	—

* See footnotes to Table 1 for calculations.

[†] The complexity values are the average ± SEM of 8, 6, and 6 determinations for 0-, 4-, and 72-hr RNA, respectively. The 0- and 4-hr values are significantly different, $P < 0.05$. The 4- and 72-hr values are significantly different, $P < 0.01$.

At 0 hr of chloroplast development, cellular RNA hybridizes with 9 kb of *Pst*-C. This value increases to 11 kb at 4 hr of chloroplast development and again to 15 kb at 72 hr. When 4-hr and 72-hr RNA are mixed and hybridized to [³H]*Pst*-C, the saturation value is the same as with 72-hr RNA alone (Table 4). Therefore the 4-hr RNA population is a subset of the 72-hr RNA population. It can be concluded that 9 kb within the *Pst*-C region yields cellular RNA transcripts throughout chloroplast development. In addition, the expression as cellular RNA of 2 kb within *Pst*-C is activated by 4 hr of chloroplast development and a further 4 kb of *Pst*-C is activated by 72 hr.

***Pst*-D and *Pst*-E Regions.** The *Pst*-D and *Pst*-E regions of the chloroplast genome are 10 kbp and 7 kbp, respectively. At all stages of chloroplast development tested, the hybridization kinetics of total cellular RNA to [³H]*Pst*-D or [³H]*Pst*-E are those of a single pseudo-first-order reaction (R_0t curves not shown). The quantitative analyses of the RNA-driven hybridization reactions to [³H]*Pst*-D and [³H]*Pst*-E are listed in Table 5. There is no significant change during chloroplast development in the size of the region of *Pst*-D transcribed.

In contrast, the transcription of the *Pst*-E region is activated between 0 and 4 hr of development and then remains constant through 72 hr of development. The results obtained when [³H]*Eco*-H is hybridized to cellular RNA from the three stages of chloroplast development are similar to those obtained with [³H]*Pst*-E (Table 5). Because the *Eco*-H region overlaps the

Table 5. Analysis of the hybridization of developing *Euglena* RNA to the chloroplast DNA *Pst*-D and *Pst*-E regions*

Probe	RNA driver, hr	R _{0t} _{1/2} obs., M-sec	Complexity, kb	R _{0t} _{1/2} calc., M-sec	Total RNA, %
<i>Pst</i> -D	0	25	2.7	0.0015	0.0060
	4	20	2.2	0.0012	0.0060
	72	9.6	2.2	0.0012	0.012
<i>Pst</i> -E	0	9.0	1.0	0.00056	0.0062
	4	52	2.2	0.0012	0.0023
	72	8.1	2.3	0.0013	0.016
<i>Eco</i> -H	0	6.6	0.8	0.00045	0.0068
	4	48	2.2	0.0012	0.0025
	72	6.8	2.3	0.0013	0.019

* See footnotes to Table 1 for calculations.

Table 6. Transcription patterns of the chloroplast genome observed during chloroplast development*

Pattern of expression	Size of transcribed regions, kb					Total
	<i>Pst</i> -A	<i>Pst</i> -B	<i>Pst</i> -C	<i>Pst</i> -D	<i>Pst</i> -E	
Continuously present	21	12	9	2	1	45
Present at 0 and 72 hr, absent at 4 hr	5	2	0	0	0	7
Absent at 0 hr, present at 4 and 72 hr	0	0	2	0	1	3
Present only at 72 hr	4	3	4	0	0	11

* Data summarized from Tables 1–5.

Pst-E region by 3.4 kbp (see figure 1 of ref. 7), it may be concluded that the 2.2 kb transcribed from *Pst*-E are located within the 3.4-kbp overlap with *Eco*-H.

DISCUSSION

In this report, investigations into the regulation of expression of the *Euglena* chloroplast genome during chloroplast development are described. By using hybridization probes prepared from isolated chloroplast DNA restriction fragments for RNA-DNA hybridization experiments, the transcription programs of specific, defined regions of the chloroplast genome were studied. The RNA-driven hybridization technique utilized is extremely sensitive. Transcripts present as only an extremely small fraction of the cellular RNA can be detected. In addition, quantitative data on the sizes of the transcribed regions and the abundances of the RNA products in the cell can be obtained. This method of treating hybridization data has been applied to the study of developmental changes in *Euglena* chloroplast rRNA transcripts (7, 9). It should be noted that the error in the quantitative estimate of the RNA abundance ("Total RNA" in Tables 1–5) is proportional to the uncertainty in the measured $R_{0t1/2}$ values. Uncertainty in the $R_{0t1/2}$ values, perhaps a factor of 2–5, is inherent both in the experimental method and in the treatment of transitions in R_{0t} curves as single kinetic components.

Several types of RNA regulatory patterns are observed for the expression of the chloroplast genome during chloroplast development. These classes of chloroplast RNA transcripts are listed in Table 6. They may be summarized as follows:

(i) The largest class of chloroplast genes are those whose transcripts are found in cells at all stages of chloroplast development. The transcripts of these genes total 45 kb in complexity and are scattered throughout the chloroplast genome (see Table 6). Contained within this "continuous" class are the three sets of chloroplast rRNA genes (14 kb within the *Pst*-A region), a region of approximately 3 kb within *Pst*-B that yields high-abundance transcripts, and 28 kb of regions scattered throughout the genome that yield low-abundance transcripts. The conclusion that chloroplast rRNA is continuously present at all developmental stages is in agreement with previous results (9) obtained by using a hybridization probe specific for these transcripts. This conclusion is also in agreement with the results of Cohen and Schiff (12), but in disagreement with other investigators (13), who have maintained that chloroplast rRNA is absent in dark-adapted cells.

(ii) A class of RNA transcripts is found that are present in dark-adapted cells but that disappear within the first 4 hr after the onset of chloroplast development. Interestingly, these transcripts are then found to reappear by 72 hr of development. The disappearance of these transcripts within the first 4 hr of chloroplast development must involve RNA degradation. Whether transcriptional repression is involved is not known. These genes are located in the *Pst*-A and *Pst*-B regions of the chloroplast genome. One kilobase of these genes located in *Pst*-B yields high-abundance transcripts, while the remaining 6 kb yields low-abundance transcripts.

(iii) A small class of chloroplast genes are observed whose RNA products are first found in the cell at the 4-hr stage of chloroplast development and are then continuously expressed through 72 hr of development. These RNA transcripts are absent from cells at 0 hr of development. The accumulation of the RNA in this class must begin during the first 4 hr of development. These genes total 3 kb in complexity and are located within the *Pst*-C and *Pst*-E regions.

(iv) A large fraction of the chloroplast RNA transcripts are not found in cells at 0 or 4 hr of chloroplast development but are found at 72 hr. The accumulation of these gene products must begin at some time between 4 and 72 hr after the onset of development. The genes in this class total 11 kb in complexity. The genes are located in the *Pst*-A, *Pst*-B, and *Pst*-C regions. High-abundance transcripts of this class are transcribed from 1.5 kb within the *Pst*-B region, while low-abundance transcripts of this class are transcribed from 9.5 kb within the *Pst*-A, *Pst*-B, and *Pst*-C regions.

In summary, hybridization studies aimed at characterizing developmental-stage-specific changes in the cellular content of chloroplast RNA transcripts have been described. Changes in the expression of isolated regions of the chloroplast genome during chloroplast development are used to define four classes of genes: (i) genes expressed as RNA throughout development; (ii) genes expressed only after the induction of development; (iii) genes expressed only late after the induction of development; and (iv) genes expressed both before the induction of development and late after the induction of development but not early after the induction of development.

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