Transcriptional organization and possible function of mustard plastid DNA regions expressed in vivo

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Summary. The structural organization of selected regions on mustard plastid DNA that hybridize with in vivo RNA has been investigated. Map positions as well as the orientations of several genes within restriction fragments of the large single copy region have been determined. Cross-hybridization with spinach gene probes shows these to include the gene for the P700 apoprotein of PSI, the genes for cytochromes b6 and f and the genes for the alpha (and possibly CFo-III) subunit of the ATP synthase complex. All except one of these genes are colinear with their mature in vivo RNA. The putative cytochrome f gene seems to be split. Northern analysis of several genes has revealed multiple RNA species, indicating a complex transcriptional organization. Transcribed regions, of unknown function, have also been located and mapped within the small single copy region of mustard plastid DNA.

Key words: Plastid DNA — Transcriptional organization — Mustard (Sinapis alba L.)

Introduction

Despite rapid progress in the analysis of the structural organization of plastid (pt) DNA (for review, see Whitfeld and Bottomley 1983), as yet comparatively little is known on the mechanisms of gene expression during plastid development. So far, studies on the developmentally regulated expression of plastid genes have mostly centered on the genes for the herbicide-binding M₄ 32,000 photosystem II (PSII) membrane protein and for the large subunit of the stroma enzyme ribulose-1,5-bisphosphate carboxy lase/oxygenase (RuBPCase). Their mRNAs are major ptDNA-encoded transcripts in many light-grown tissues, but are reduced or entirely absent in dark-grown tissues (Bedbrook et al. 1978; Smith and Ellis 1981; Thompson et al. 1983; Sasaki et al. 1984; Richter 1984). By hybridization of plastid RNA to DNA fragments representing almost the entire mustard (Sinapis alba) ptDNA molecule, we have recently located additional ptDNA regions that are expressed differentially in light-grown versus dark-grown tissue (Link 1984a). This work provided evidence that at least three classes of ptDNA regions can be distinguished with regard to their mode of expression during plastid development, i.e. constitutively expressed regions, light-induced regions, and transiently expressed regions. However, with the exception of the ribosomal RNA genes (Przybyl et al. 1984) and of the structural genes for the M₄ 32,000 PSII protein and the large subunit (LS) of RuBPCase (Link and Langridge 1984), the precise map position and coding function of most mustard ptDNA genes remained unknown. To provide information on the location (and expression) of additional genes, we have now analysed various cloned mustard ptDNA regions by nuclease S1 analysis and Northern hybridization. Several of the mapped mustard ptDNA genes have been tentatively identified by hybridization with probes representing spinach plastid genes of known coding function.

Material and methods

Materials. Restriction endonucleases, \textit{E. coli} DNA polymerase I holoenzyme and large fragment were obtained from Gibco/BRL. Nuclease S1, \textit{E. coli} RNA polymerase, and T4 polynu-
S1 mapping. DNA fragments (10 ng) were hybridized in solution to plastid RNA (50 μg), and digested with 100 units nuclease S1 (Berk and Sharp 1977) essentially as described previously (Link and Bogorad 1980; Link 1981). S1-protected DNA fragments were sized in 1.2% alkaline agarose gels, blotted to nitrocellulose, and detected by hybridization with 32P-copyRNA probes.

S1 mapping using 5' or 3' end labelled DNA fragments (Weaver and Weissmann 1979) was carried out essentially as described by Link and Langridge (1984).

Northern analysis. Plastid RNA (20 μg) was denaturated in 50% formamide, 6% formaldehyde, and 10 mM MOPS pH 7 (Morpholinopropanesulfonic acid) at 65 °C for 15 min. Electrophoresis was in 1.2% agarose gels containing 6% formamide, 20 mM MOPS, 5 mM sodium acetate and 1 mM EDTA (Lehrach et al. 1977). RNA was then transferred to nitrocellulose (Thomas 1980) and hybridized with nick-translation ptDNA fragments.

Results

Figure 1 presents a physical map of the 157 kb ptDNA molecule from mustard (Sinapis alba) (Link et al. 1981). Indicated are the positions of the genes that had been previously located and identified (genes for RubCase LS and Mr 32,000 PSII protein: Link 1981; rRNA genes: Pryzbyl et al. 1984) and of those genes defined by the present mapping analysis. The precise location and structural organization of these additional transcribed regions is shown in detail in Figs. 2–6. Several of these genes were tentatively identified by hybridization to spinach ptDNA probes representing genes with known coding function (see Table 1).

Mapping of transcribed regions on cloned mustard ptDNA fragments

S1 mapping (Fig. 2B) and Northern hybridization (Fig. 2D) show that the 7.3 kb EcoI region (insert of pSA244) within ptDNA fragment Pst3 (Fig. 2A) contains one single transcribed region which is colinear with a 5.1 kb RNA. XhoI cuts the transcribed region into the 3.6 kb major portion within fragment XhoI/EcoRI4.4 (Fig. 2B, lanes e, f) and a 1.5 kb minor portion within EcoRI/XhoI2.9 (lanes g, h). Among the fragments generated by digestion with EcoRI and BamHI, intragenic fragments such as BamHI/BamHI1.9 are protected against S1 digestion over their entire length (lanes c, d and additional data not shown). The right-hand border fragment EcoRI/BamHI1.0 gives rise to an S1-resistant region 550 nucleotides in size (lanes a, b). The left border of the transcribed region is defined by the 0.26 kb protected fragment within BamHI/BamHI1.45 (Fig. 2C). Since no other transcripts originating from this region are detected, the protection of the 5' label against S1 nuclease digestion indicates that the