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Chloroplast promoters from higher plants

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ABSTRACT

This survey compiles 60 chloroplast promoter sequences from higher plants published to date and compares them with these sequences from procaryotic systems. The current evidence demonstrates that structurally defined chloroplast promoters are, in most cases, functionally active in initiating gene expression in chloroplasts.

INTRODUCTION

The transcriptional cycle consists of three main steps: initiation, elongation and termination. The initiation of transcription is a crucial stage at which gene expression can be regulated by the interaction of RNA polymerase with the promoters. The promoter is defined as a region of DNA involved in the binding of RNA polymerase to initiate transcription. In procaryotic systems, with the notable exception of the nif genes in Klebsiella (1), promoters consist of two regions of conserved sequences, located about 10 and 35 bp upstream of the transcription startpoint and separated by an optimal distance of 17 bp (2,3). These characteristics, together with some secondary sequence conservation in the region upstream of the primary promoter (4), are major determinants of promoter activity.

After analyzing the DNA sequence of 112 well-defined promoters, a consensus sequence of procaryotic promoters was established (2,3,4). The AT-rich sequence around the -35 region has often been referred to as the recognition site and the sequence around the -10 region as the Pribnow box (5). Within the -35 region (TTGACA), the trimer TTG is strongly conserved, appearing at a frequency of 82, 84, and 79% for each base respectively (3). At the -10 region (TATAAT), the first TA pair and the last T are also highly preserved.

Generally, there is one promoter per gene. Sharing of promoter by more than one gene also occurs. On the other hand, there are genes which have more than one promoter (6-10). A working model for the function and regulation of multiple promoters of stable RNA has been proposed (11,12).

TABLE I: COMPILATION OF CHLOROPLAST PROMOTERS FROM HIGHER PLANTS

ORGANISMS AND GENES		PROMOTER SEQUENCES -35	PROMOTER SEQUENCES -10	REF.
E. Coli Consensus Sequence		TTGACA	TATAAT	2
(A) Structurally and functionally defined:				
N. tabacum	rbcL	AAGTAAAAAAGAAAAATTGGG	TTGCGCTATATATATGAAAGAGTA	TACAATAATGATGTATTTGGCAAATC 21
N. otophora	rbcL	AAGTAAAAAAGAAAAATTGGG	TTGCGCTATATATATGAAAGAGTA	TACAATAATGATCTATTTGGCAAATC 56
N. tabacum	psbA	ATAGATCTACATACACCTTGG	TTGACACGAGTATATAAGTCATGT	TATACTGTTGAATAAAAAGCCTTCCA 42
N. debneyi	psbA	ATAGATCTACATACACCTTGG	TTGACACGAGTATATAAGTCATGT	TATACTGTTGAATAAAAAGCCTTCCA 31
N. tabacum	atpB	TCAGGTTCGAATTCCATAGAA	TAGATAATATGGATGGGATTGTC	TATAATGATAGACAAATGAAAGACTT 33
N. otophora	atpB	TCAGGTTCGAATTCCATAGAA	TAGATAATATGGATGGGATTGTC	TATAATGATAGACAAATGAAAGACTT 47
N. tabacum	5S rRNA	GGTGTCCCCTCCAGTCAAGAA	TTGGGGCCTCACAATCACTAGCCAA	TATGCTTTTCTCTCATGCCTTTCTTC 22, 38
	16S rRNA	AGTTGTTCAAGAATAGTGGCG	TTGAGTTTCTCGACCCTTTGACT	TAGGATTAGTCAGTTCTATTTCTCGA 23
	$tRNA^{Gly}$	TGATTACCACAATTCCCCTGT	TCGACAAAAGTTGCATTTGTA	TACAATAATCGGATTGTA 30
	?	GCTGTGTTCGGGGGGGAGTTA	TTGTCTATCGTTGGCCTCTATGG	TAGAATCAGTCGGGGGACCTGAGAGG 36, 38
	?	CGCACCATCGAAAACCGAATT	TTGCTGGTGGCTAACGTATACCCCTG	TAGCGTAACGTGACGGACGTAACCAC 38
Maize	rbcL	AAATAAAGATTAGGGTTTGGG	TTGCGCTATATCTATCAAAGAGTA	TACAATAATGATGGATTTGGTGAATC 20
	atpB	AAATACTAAGAAAATTCTCTG	TTGACAGCAATCTATGCTTCACAG	TAGTATATATTTTGTATATCGAAGTC 39, 58
	16S rRNA	ATGGATAGGAGGCTTGTGGGA	TTGACGTGATAGGGTAGGGTTGGC	TATACTGCTGGTGGCGAACTCCAGGC 26
	$tRNA^{Val(1)}$	TCCTATTTTCGATAGGACCGG	TTGACAATTGAATCCAATTTTTCCCAT	TATTTGACTGTCCATAATAGTGCGGA 26
	$tRNA^{Val(2)}$	AAGCCCGGAGGAAGAGTGGCC	TTGCGTTTCTCGCCCCTTTGCCT	TAGGATTCGTTAATTCTCTTTCTCGA 26
	$tRNA^{His}$	TCAGAATAAATAGAATAATAA	TGAATGGAAAAAAGAGAAAATCCT	TTAGCTGGATAAGG 40
Spinach	rbcL	AAACCAACGGTTACGGTTGGG	TTGCGCCATATATATGAAAGAGTA	TACAATAATGATGTATTTGGCGAATC 19
	atpB	TAAATAATTCGAAATTTACTC	TTGACAGTGGTATATGTTGTATATG	TATATCCTAGATGTGAAAATATGC 58
Wheat	rbcL	AGGATTAGGAATTAATTTGGG	TTGCGCTATATCTATCAAAGAGTA	TACAATAATTATGGATTTGGTAAATC 47
	atpB	AAATACTAATAAAATTCTTTG	TTGACAGCAATCTATGCTTCACAG	TAGTATATTTTGTATATATCGAAGTT 47
Mustard	psbA	ATCTTATCCATTTTACATTGG	TTGACATGGCTATATAAGTCATGT	TATACTGTTCAATAACAAGCTCTCAA 45
Pea	rbcL	CTCAAAAAAAAAACGGTTGGG	TTGCGCCATACATATGAAAGAGTA	TAGAATAATGATGTATTTCCCAAA 14, 58
	atpB	AAAAGATATTCTTGACC	TTGACAGTGATCTATGTTGTATATG	TAAATCCTAGATGTAAAAATCGGCAG 58
(B) Structurally defined:				
N. tabacum	$tRNA^{Asn}$	AAGGGTATTAAATGAATGGAA	TTGGGATATAGGATGGAA	TATAATGAAATAGAGCCACTTTGAGG 27
	$tRNA^{His}$	AAAGAAGAGCTATATTCGAAC	TTGAATCTTTTGTTTTCTAATTTA	AATAATGTAAAAACGGAATGTAAGTA 42
	$tRNA^{Met}$	TGTATAAATGGGCTATTCTAT	TTGTACAGATAGGGTGGAGGGGCGCA	TTTAATCCTTGTTTATCTATTAGTTT 46
	$tRNA^{Pro}$	CGGGTTCTGTATTTATATATT	TTGTATATAATTGTATATAAGTATTTTCTA	TATAATCTATAAGAGAAGTCTTTTCC 46
	$tRNA^{Trp}$	ATCAATTGAGATCGCCTCAAA	TTGGACATAATCTTTGATTTT	TATCATGCTATTCTAGTATATGCATA 46
	$tRNA^{Arg(1)}$			TACAATTCCAAAAATTCTTTCACATC 30
	$tRNA^{Arg(2)}$			TAAAATACGAAAAAAAAATCAGAATG 30
	$tRNA^{Val(1)}$	GCTCAAAGAGATCAAAGATTG	TTGATGTTGGATCATGGAATATT	TATCTTGACAAGAATTTATCTACATG 24
	$tRNA^{Val(2)}$	TTGGATCATGGAATATTTATC	TTGACAAGAATTTATCTACATGA	TAAAATATGTATCACAAGCACTA 24
	CS19	CCCCTTGGGGTTATCCTGCAC	TTGGAAGAAGAAGTAGAAAAAGGAATAAA	TATAGTGATAATTTGATTCTTCGTCG 28
	$tRNA^{Gly(1)}$	TGATTACCACAATTCCCCTGT	TCGACAAAAGTTGCATTTGTA	TACAATAATCGGATTGTA 30

	$tRNA^{Gly(2)}$	AGAGAATATGTGTCCCGGCAC	TGCACAAAAAAGATCCGGTTATA	TATCATATATGTGGGTACATATTGTG	46
	CF. III	CCCTTCTAGATGTTTCGACGC	TTGATTCTCGAATAGGATTGAATC	TAAGATGAATGCTTGGTTTACGTTAT	53
Maize	$tRNA^{Leu(1)}$	TAATGAATTCAATGA	TTCAAAAAAAACTAAGAGATGGA	TTAAATTATACAAGGAATCCTGGTTT	34
	$tRNA^{Ser(1)}$	GAGTTAGTAGATCATTTCATA	TAGCTATGTTCTATTTGTAGGAA	TAAAATAGGGATTGGGCTGT	29
	$tRNA^{Ser(2)}$	CAGGAATACGAAAACTCGCTA	TTCACTCAGTTTATTTTCCATAA	TAAGATTATGTA	25
	$tRNA^{Val}$	TGGCATTAGAGAATATTCATC	TTGACAAGAAATTATCTATATGT	TAAGATATCTCTGAC	29
	$tRNA^{Leu(1)}$	AAGACTCCACCT	TTGTCATATATTCCATATATCACA	TTCGATAGATATCATATTCATGGAAT	25
	$tRNA^{Leu(2)}$	AGACTCCACCTTTGTCATATA	TTCCATATATCACATTCGATAGA	TATCATATTCATGGAATACGATTCAC	25
	$tRNA^{Met}$	CATACCAATAACGGAGCGGTA	TTGCTTATAAAAAGGATTCAATC	TATAATCGATCGAAGTAATGGGGCTT	25
	$tRNA^{Phe}$	TTGATTTTTTAGTCCCTTTAA	TTGACATAGATGCAAATACTTTAC	TAAGATGATGCACAAGAAAGG	25
	$tRNA^{Thr(1)}$	CTATCTAAGTGGAACTTCCAA	TTTAGAACTAGTTAATAAC	TAAGATTAATAATTAAGATCTGACAT	25
	$tRNA^{Thr(2)}$	GAACTTCCAATTTAGAACTAG	TTAATAACTAAGATTAATAAT	TAAGATCTGACATTTTACAGATTCCC	25
	D2(1)	TAATATAGAAAACGATTTTTT	TTGATTTCACGAACAAGATTCAAGAA	TAATCTTATTTGATAAAAGCAGAGTA	55
	D2(2)	GTTAATGGATTTGACCTAGAT	TAGATATCAATCGACAAAAAAA	TAATTTTTCTATTCGAAACCCAGTCG	55
Spinach	P680	AGACGATGCTATCAACTCCGA	TTGCGTATTGCTACTTATCGAGTA	TAGAATAGATTTGTTTCTCTTTGTTC	35
	psbA	ATAGATCTCACTAGATATTGG	TTGACACGGGCATATAAGGCATGT	TATACTGTTGAATAACAATCTTTAA	31
S. nigrum	psbA	ATAGATCCAGATACAGCTTGG	TTGACACGAGTATATAAGTCATGT	TATACTGTTGAATAACAAGCCTTCCA	49
Soybean	psbA	TACTATGGATATTGGTATTGG	TTGACACTGGTATATAAGTCATGT	TATACTGTTGAATAACAAGTCCTCAA	44
Duckweed	16S rRNA	ATGAATAAGAGGCTCGTGGGA	TTGACGTGATAGGGTAGGGATGGC	TATATTGCTGGGAGCCGAACCTCCAG	41
	5s rRNA	GGTGTCCCTTCCAGTCAAGAA	TTGGGGCCTCACAATCACTAGCCAA	TATGAATATGCTTTTCTCTCATGACT	43
Broadbean	rbcL	GACTCAAAAAAAACGGTTGGG	TTGCGCCATACATATGAAACAGTA	TAGAATAATGATGTATTTGCCAAATC	50
	atpB	AAAGTTCAGGTTCGAATTACA	TAGATAATATAGATAGTATTGTC	TATAATCTAGAATGATAAACAAATGA	50
	$tRNA^{Glu}$	GAATCATATCATTCCATTATA	TTGACAATTTCAAAAAACTGTTCA	TACTATGAACATAGTAGAATGGAAAT	54
	$rRNA^{Thr(1)}$	TGTACTAAACTCATCTTCATA	TTGGCTGATTCCGTATTGGGGAA	TTTACTCAAACGCC	54
	$tRNA^{Thr(2)}$	ATATATATCTATTTCGTCAGA	TTGATATACCAATTTTGTATATATC	TATTTTGTATATCTATCTATAATAAT	54

CHLOROPLAST PROMOTERS

The chloroplast is probably procaryotic in origin and therefore possesses the procaryotic type machinery for protein synthesis (13-18). Many chloroplast genes and their 5'-flanking regions have been sequenced and studied (19-60). There are conserved promoter sequences in front of many chloroplast genes analyzed to date. Table I lists two groups of chloroplast promoters. The inclusion of promoters in the first group (A) was based on both structural and functional considerations. Chloroplast promoters must resemble those of procaryotic promoter in structure and satisfy one of the functional criteria. (a) These structures are protected by *E. coli* RNA polymerase against DNase digestion. (b) These structures are determined by S1 mapping to be located in the 5' flanking region of a gene. And (c) these structures are active in initiating gene expression in either heterologous or homologous system. All

the promoters included in the second group (B) are defined structurally only. For example, a promoter can be identified by locating DNA sequences matching the structure of procaryotic consensus sequence promoters proximal to a chloroplast gene. As can be seen from Table I, most chloroplast promoters identified thus far fall into the second group (B). The alignment of the promoters in Table 1 was based on the format and considerations given by Hawley & McClure (3) to maximize the homology of both the -35 and -10 regions. In order to align promoters with different spacing, two breaks were placed in the sequences; one immediately after the -35 and one immediately before the -10 regions. In addition to the -35, -10 regions and the spacer, 20 more bases were included at each end in most cases when they are available to have a total of about 70-80 bases for each promoter.

In both groups (A & B) these promoters contain two conserved hexamers separated by a short stretch of about 17 bp. The first hexamer is TTGACA resembling the -35 region of procaryotic promoter sequences, in which the trimer TTG is highly conserved. In the second hexamer, the conserved sequence of TATAAT is identical to the -10 region (5). Within this hexamer, three bases (TA---T) are highly conserved. The first TA pair was present in all but few cases, in which the pair was either AA or TT (Table I). Similar to the procaryotic promoters, the last "T" in this hexamer is also highly conserved, appearing in all but few of the 60 promoters compiled (Table I). The -35 and -10 regions are separated by 11-24 bases which is very close to the allowed procaryotic spacing of 15-21 bp (2,3). Overall, the distribution of bases in each position in the -35 and -10 regions of these chloroplast promoters is statistically similar to that of procaryotic promoters (Table II).

As in *E. coli*, secondary or tertiary promoters in the region upstream from the primary promoter sites for stable chloroplast RNA genes were also found (Table I). For example, there are two promoter sites reported for many tRNA genes (24,25). In most cases, the secondary promoters have -35 and -10 regions separated by 11-22 bp, and in a few cases only the individual isolated -10 regions were identified (30). The existence of multiple promoter sites was reported for maize and duckweed rRNA genes (26,41). Conversely, sharing of promoter by more than one gene also exists. The rRNAs (23), some tRNAs (51) and the β and ε (32) genes are co-transcribed.

The functional assay of chloroplast promoters (38,47, 52) in the pKO1 system established a certain structure-function relationship of the sequences tested. Using plasmids with sequential deletions in the 5'-flanking region of the psbA gene, and a homologous chloroplast extract, Link (57) demonstrated

TABLE II Distribution of Bases at each Position in Promoters of Chloroplast Genes

POSITION	DISTRIBUTION											
	T	T	G	A	C	A	T	A	T	A	A	T
A	0	5	2	35	7	30	1	55	14	36	40	1
T	58	49	1	4	13	12	59	5	27	7	7	56
G	0	2	51	6	14	7			9	12	2	1
C	0	2	4	13	24	9			10	5	11	2
Chloroplast (%)*	100	84	88	60	41	52	98	92	45	60	67	93
Procaryotes (%)**	82	84	79	64	54	45	81	95	44	59	51	96

* Calculated from Table I

** Calculated from reference 3

that the upstream region containing the sequences of TTGACA and TATACT, which resemble the procaryotic -35 and -10 regions, is required for efficient *in vitro* transcription. Employing a similar technique Chua and his co-workers (48) have demonstrated that increasing the distance between the -35 and -10 regions of maize rbcL promoter from 18 pb to 20 bp with an AT base pair insertion reduced the level of transcription drastically. It is clear that the -35, the -10 regions and the distance between them are involved in the modulation of the efficiency of chloroplast promoters. The evidence obtained from the functional assays in the homologous system demonstrated that some structurally well-defined chloroplast promoters are indeed functionally active promoters (52, 60). Therefore, this survey can be considered as a preview of the nature and properties of chloroplast promoters from higher plants.

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