Nucleotide sequence of soybean chloroplast DNA regions which contain the *psb* A and *trn* H genes and cover the ends of the large single copy region and one end of the inverted repeats

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ABSTRACT

The soybean chloroplast <u>psb</u> A gene (photosystem II thylakoid membrane protein of Mr 32 000, lysine-free) and the <u>trn</u> H gene (tRNAHig), which both map in the large single copy region adjacent to one of the inverted repeat structures (IR1), have been sequenced including flanking regions. The <u>psb</u> A gene shows in its structural part 92% sequence homology with the corresponding genes of spinach and <u>N</u>. <u>debneyi</u> and contains also an open reading frame for 353 aminoacids. The aminoacid sequence of a potential primary translation product (calculated Mr, 38 904, no lysine) diverges from that of spinach and <u>N</u>. <u>debneyi</u> in only two positions in the C-terminal part. The <u>trn</u> H gene has the same polarity as the <u>psb</u> A gene and the coding region is located at the very end of the large single copy region. The deduced sequence of the soybean chloroplast tRNAHis is identical with that of <u>Zea mays</u> chloroplasts. Both ends of the large single copy region were sequenced including a small segment of the adjacent IR1 and IR2.

INTRODUCTION

We have recently mapped the <u>psb</u> A gene on the soybean (<u>Glycine max</u>.) chloroplast genome in close vicinity of one of the inverted repeat regions (1). This gene codes for the so-called Mr 32 000 rapidly labeled photosystem II thylakoid membrane protein (2,3) which may be involved in the binding of urea and triazine herbicides (4,5). Zurawski et al. (6) sequenced the <u>psb</u> A gene region from <u>Spinacia oleracea</u> and <u>Nicotiana debneyi</u> chloroplast DNA and they observed in both cases identical open reading frames for 353 aminoacids equivalent to a protein of Mr 38 950. The size of this primary translation product surpasses the size of the rapidly labeled thylakoid membrane protein (Mr 32 000 to 36 000); the authors therefore argued that translation might start at a second ATG in the same reading frame, reducing thereby the size of the translation product by about 4 000. On the other hand, the total sequence identity of the reading frame in both types of chloroplast DNA strongly suggested that the entire reading frame must be functional.

Nucleic Acids Research

In view of these open questions and considering the functional importance of this gene product it seemed warranted to sequence the <u>psb</u> A region of the soybean chloroplast genome. Representatives of the legume family are known to contain chloroplast genomes which have undergone relative to other angiosperm chloroplast genomes considerable DNA rearrangements (7,8). Therefore, size and fine anatomy of the soybean chloroplast <u>psb</u> A gene region might be different from those reported and answer some of the open questions.

Swamy and Pillay (9) recently identified soybean chloroplast $tRNA^{His}$ without, however, mapping the corresponding gene. In case of spinach the <u>trn H</u> gene maps between the <u>psb</u> A gene and the inverted repeat (10). We included in our sequence studies the corresponding DNA segment and furthermore determined the beginning of the inverted repeats by sequencing the corresponding DNA region on the other side of the large single copy region. This allowed to exactly position both the <u>psb</u> A and <u>trn</u> H gene relative to one of the inverted repeats, which are structural hallmarks of most higher plant chloroplast genomes.

MATERIALS AND METHODS

Isolation of soybean chloroplast DNA, restriction sites analysis and mapping of the psb A gene on the circular chloroplast genome have been described (1). For a more detailed restriction site mapping and sequencing of the relevant region, we cloned HindIII fragments of total chloroplast DNA into pBR322 and tested the clones with both, a nick-translated (11) HpaII fragment obtained from the clone pSoc B511 (1,3) which carries 330 nucleotides of the spinach chloroplast psb A gene (probe a) and a HpaII-SalI fragment (850 bp) which carries the 3' end of the psb A gene including about 200 bases of the adjacent inverted repeat (probe b). A HindIII fragments of 2.8 kb (HindIII-J) interacted only with probe a, a HindIII fragment of 1.4 kb (HindIII-0) interacted with probe a and b and a HindIII fragment of 9.0 kb (HindIII-B) interacted only with probe b. Further mapping experiments allowed to place the three HindIII fragments as shown in Fig. 1A,D. For the sequencing experiments we used the entire fragments HindIII-0, the HindIII-SmaI subfragment of HindIII-J (0.8 kb) and a HindIII-PstI subfragment (3.9 kb) of HindIII-B (Fig. 1,A,D). DNA fragments were isolated and purified as described (12). For DNA sequencing we used both current methods (13,14) and as specified (12). Enzymes were purchased from Boehringer-Mannheim and New

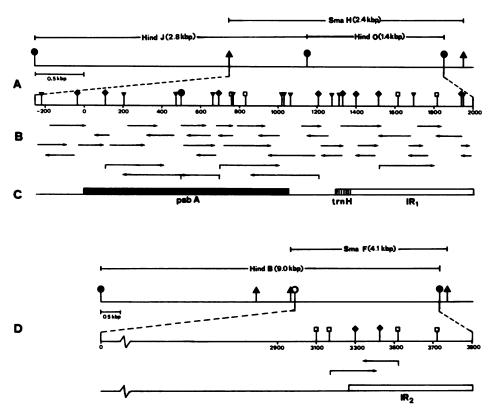


Fig. 1. Restriction sites map and strategy used to sequence the psb A and trn H gene region of the soybean chloroplast DNA. A. Restriction sites of SmaI-H fragment (1). B. Arrows represent portions of the 5' end labeled fragments from which unambiguous sequences could be established (13); \longrightarrow RNA-like strand, \longleftarrow coding strand; arrows with a short vertical line indicate regions sequenced according to (14). C. Location of structural parts of the psb A gene, trn H gene and parts of the inverted repeat [IR1]. D. Restriction sites map of SmaI-F fragment and position of parts of the inverted repeat [IR2]. \P HindIII, \P SmaI, \P Sau3A, \P HinfI, \P HaeIII, \P PstI. Numbers on scales in A and D refer to nucleotide sequence position given in Figs 2 and 3.

England Biolabs and used following the instructions of the supplier. $^{32}P-ATP$ was from Amersham.

RESULTS

1. Sequence analysis of the psb A gene

In Fig. 1 we show a stretch of the soybean circular chloroplast DNA which carries the psb A gene. In Fig. 2 we give the nucleotide sequence of the

-260 -248 S.O. CCATTETT TAATAC TGAAAT CAT ATTCTTT AG TITCTTITTC G. R. CCACTAGAATTAATATATCEAAATTCTATATATAGAATTAT AGCG N. J. CCACTAGE ATAT CHARACTERA ATTCTCTGTAG G AAGTC -220 -201 -180 -160 -140 -120 AAAAA CTGA TA GA T CTC ACTAGATATTGGTTGACAQGGG AATTCACTTCCAT TTEAC <u>, htter/kganantanetentfittantganatgagetahnangstantteggatanatetanatangkgeataetetaetaetaetaetaetaetaetaetegeta</u>etgettgaetg COTATTITTCATC TAAAATT TGAATA GA T CTA CATACACCETGGTTGACAGGAG AACTTCAT teTTGACa -10 -100 -88 - 60 -48 -29 CATATAACE ATGTTATACTGTTGAATAACAAT TATAACHEATCTTATACTSTTGAATAAEAAC TCCTCAAATTITCTAATTCTAGATAATTTTGGTGCTTGGGAGTCCCTGATGATTAAAT CAACCAAGATTTTACC ТАТАТААС<mark>ИС</mark>АТСТТАТАСТСТТСВААТАА<mark>А</mark>АААСССТТССТТТСТА <u>ТТТТТАТТТСТА</u>САААААСТАСТ<mark>Т</mark>ССТТСССАСССТСАТСАТТАААТ CAACCAAGATTTTACC tg TAthaT 98 τε 1 T ATG ACT GCA ATT TTA GAG AGA CGC GAG AGC GAA AGC CTA TGG GGT CGC TTC TGT AAC TGG ATA ACC AGC ACC GAA AAT CGT CTT TAC ATT тε т Het Thr Ala lie Leu Clu Arg Arg Glu Ser Glu Ser Leu Tro Gly Arg Phe Cys Asa Trp lle Thr Ser Thr Glu Asa Arg Leu Tyr lle 18 28 30 180 C C T A A C C Т GGA TGS TTT GGT GTT TTG ATG ATT CCT ACT TTA TTG ACC GCC ACT TCT GTA TTT ATT GCT GCT TTT ATT GCT GCC CCT CCA GTA GAT ATT C C G A тсс T C Gly Trp Fhe Gly Val Leu Met lie Pro Thr Leu Leu Thr Ala Thr Ser Val Phe lie Ala Phe lie Ala Ala Pro Pro Val Asp lie 44 58 60 279 C C т 7 ٤. G GAT GGT ATT CGT GAG CCT GTT TCT GGA TCT CTA CTT TAT GGA AAC AAT ATC ATT TCT GGT GCC ATT ATT CCT ACT TCT GCG GCT ATA GGT λ λς ς 7 C Asp Gly 11e Arg Glu Fro Val Ser Gly Ser Leu Leu Tyr Gly Asm Asm 11e 11e Ser Gly Ala 11e 11e Pro Thr Ser Ala Ala 11e Gly . 74 90 368 а г ТТ C c TTG CAC TTT TAT CCT ATT TGG GAA GCG GCA TCT GTT GAT GAA TGG TTA TAC AAC GGC GGT CCT TAT GAA CTA ATT CTT CTA CAC TTC TTA AT CAC C т Leu His Phe Tyr Pro lle Trp Glu Ala Ala Ser Val Asp Glu Trp Leu Tyr Asn Gly Gly Pro Tyr Glu Leu Ile Val Leu His Phe Leu 194 110 126 450 тт т C C L C C A CTT GGT GTA GCT TAC TAC ATG GGG CGT GAG TGG GAA CTT AGT TTT CGT TTG GGT ATG CGT CCT TGG ATT GCT GTT GCA TAT TCA GCT CCT C T T G C C λ Leu Gly Val Ala Cys Tyr Het Gly Arg Glu Trp Glu Leu Ser Phe Arg Leu Gly Het Arg Pro Trp Ile Ala Val Ala Tyr Ser Ala Pro 138 140 150 540 C CACTA Т C C GTT GCA GCC GCT ACT GCT GTT TTC TTG ATC TAT CCT ATT SGA CAG GGA AGC TTT TCA GAT GGT ATG CCT CTA GGA ATT TCA GGT ACT TTC T C À C À C T À T T с т Val Ala Ala Ala Thr Ala Val Phe Leu lie Tyr Pro Ile Gly Gin Gly Ser Phe Ser Asp Gly Met Pro Leu Gly lie Ser Gly Thr Phe 163 178 180 638 C C C C c ANT TIT ATG ATT GTA TIT CAG GCT GAG CAT ANT ATT CTT ATG CAT CCA TIT CAC ATG TTA GGT GTA GCT GGT GTA TTC GGC GGC TCC CTA τ τ τ τ τ σ С C Asn Phe Het lle Val Phe Gin Ala Giu Nis Asn lle Leu Met His Pro Phe His Met Leu Giy Val Ala Giy Val Phe Giy Giy Ser Leu 178 288 210

720 C 7 TTE AGT GCT ATG CAT GGT TEC TTG GTA ACT TCT AGT TTG ATC AGG GAA ACC ACA GAA AAT GAA TCT GCT AAT GAA GGT TAC AGA TTT GGT Phe Ser Ala Met His Gly Ser Ley Val Thr Ser Ser Leu Ile Arg Glu Thr Thr Glu Asn Glu Ser Ala Asn Glu Gly Tyr Arg Phe Gly 220 238 240 818 Ŧ c т т ٢ CAN GAG GAN ACC TAT NAT ATT GTA GET GET CAT GGT TAT TTT GGE CGA TTG ATE TTE GAN TAT GEA AGT TTE ANE NAT TET CGT TET C C C T C C Gin Giu Giu Giu Thr Tyr Asn lie Val Ala Ala His Giy Tyr Phe Giy Arg Leu lie Phe Gin Tyr Ala Ser Phe Asn Asn Ser Arg Ser 258 268 270 900 C TT 1 TTA CAT TTC TTC TTC GCT GCT GGC GCT GTA GGT ATT TGG TTT ACC GCT TTA GGT ATC AGC ACT ATG GCT TTC AAC TTA AAT GGT TTC C C C Leu His Phe Phe Leu Ala Ala Trp Pro Val Val Gly Ile Trp Phe Thr Ala Leu Gly Ile Ser Thr Met Ala Phe Asa Leu Asn Gly Phe 284 298 308 C T т T Т ANT TTE ANE CAN TEE GTA GTT GAT AGT CAN GGT EGT GTA ATT ANT ACE TEG GET GAT ATT ANE EGA GET ANE ETT GGT ATG GAN GTA T c c T C Т т àsn Phe Asn Gin Ser Val Val Asp Ser Gin Gly Arg Val 11e Asn Thr Trr Ala Asp 11e 11e Asn Arg Ala Asn Leu Gly Met Giu Val 316 326 330 1056 1876 C C TTA CA AATTTCATTTTAGCT TAGTGT ATG CAT GAA CGT MAT GCT CAT MAT TTC CCT CTA GAT CTA GCT GCG ATC GAC GCT CCA TCT ATT MAT GGA TAM MATTTGGATCTTAMGG TAGATT **C** C C T GATCCC AGCCTAGTCT CA A Met His Glu Arg Asn Ala His Asn Phe Pro Leu Asp Leu Ala Ala Ile Asp Ala Pro Ser Ile Asn Gly TER 344 358 1096 1110 1130 1150 1170 AGATGAGTTAGT GAAAGTAAAGGGGCAGTE CEGA TITETTGACAAAAACAAGAAATCGGTTATTGCTCCTTTACT AGTAC TACTTATABATA AGATG TITTT GARAGTAAAAGG CAATA TCAACTTTTTTCA TATTGCCCCCTTTACTITTATTTATTTGATTAGTAATCTTTTTATTTATAAATA ATAGGAGGTTTTGAAAAGAAAG GAG CAATAATCAT TITCITETTCTATCAAGAGGG TECTATTE TEETTT TTT CITITATIT Fig. 2. Nucleotide sequence of the psb A gene and flanking regions. Only the

RNA like strand is given starting with the 5' position. Aligned with the soybean chloroplast <u>psb</u> A gene region (G.m.) are the corresponding sequences of <u>Spinacia oleracea</u> (S.o.) and <u>Nicotiana debneyi</u> (N.d.); within the structural part only deviations from the soybean sequence are given; within the flanking parts the entire sequences are shown and aligned such as to maximize sequence homology. Homologous regions are boxed. The deduced aminoacid sequence is given; the first methionine of the open reading frame is taken as position 1. Potential regulatory sites in the 5' flanking and a potential stem-loop structure in the 3' flanking part are underlined. Prokaryotic promoter consensus sequences for the -35 and -10 region (15) are given.

<u>psb</u> A gene and its flanking regions along with the deduced aminoacid sequence for the structural part of the gene. For comparative reasons we add the nucleotide sequences of the corresponding parts of the <u>S</u>. <u>oleracea</u> and <u>N</u>. <u>debneyi</u> chloroplast DNA. The results of this comparative study can be summarized as follows : 1) The coding part of the soybean <u>psb</u> A gene is identical in length to that of S. oleracea and N. debneyi, i.e., the reading frame is also open for a maximum of 353 aminoacids. 2) The soybean psb A gene diverges in its coding part at 73 (78) nucleotide positions from the S. oleracea (N. debneyi) gene. 3) With only three exceptions, the nucleotide differences occur in the wobble position, and only two mutations lead to a change in the aminoacid composition : aspartic acid replaces glutamic acid (position 347) and isoleucine replaces threonine (position 351). 4) Codons for lysine are absent in both cases. 5) About 130 nucleotide positions of the 5' flanking part are highly conserved, the sequence homology being in the range of 80 to 85%. This region contains potential promotor sites. Within this region the soybean sequences shares once a pentanucleotide gap with the N. debneyi sequence and once a heptanucleotide gap with the S. oleracea sequence. 6) Within the positions -130 to -271 sequence homology is very low especially due to multiple short insertion/deletions. 7) The 115 positions of the 3' flanking part are homologous, respectively, to 63% and 57% with the S. oleracea and N. debneyi counterparts. This includes gaps required for maximal sequence alignement. 8) A stem and loop structure with 21 basepairs can be formed [positions 1094 - 1115 ~ 1121 - 1143] similar to but not identical with the stem and loop structures proposed for the corresponding sequences of S. oleracea and N. debneyi (6). They may serve as transcription termination signals.

2. <u>Sequence analysis of the trn H gene and of a segment overlapping the large</u> single copy region and the inverted repeats

The circular soybean chloroplast genome contains two inverted repeats which separate a large (84 kb) from a small (24 kb) single copy region (1,8). The <u>psb</u> A gene maps close to one of the inverted repeats (1) the identified \underline{trn} H gene (9) was not located yet. We anticipated, however, that the \underline{trn} H gene would map between the <u>psb</u> A gene and the IR1, as seen in other angiosperm chloroplast genomes (10). In order to exactly locate the relative map positions of these genes and study the fine anatomy we sequenced the gap between the <u>psb</u> A gene and the beginning of the IR1, including a small part of the IR2. To identify the beginning of the IR1 it was necessary to sequence the corresponding DNA segment on the other side of the large single copy region which was mapped as shown in Fig. 1D. The sequence results are given in Fig. 3. The gap between the terminator codon of the <u>psb</u> A coding region and the first nucleotide of the inverted repeat is 319 positions (nucleotide positions

1200	
TCTTTTTATTATAAATATTTATACATAAGTTTTTGATTTCTTTC	Г
1250 1300	
AAAAGGAAAAAAGAATGATAACGAACGAAAGGATAGAATTTTATATATA	Ē
	-
/ ** *	
1350	
GGATGTAGECAAGTGGATCAAGGCAGTGGATTGTGAATCCACCATGCGGGGTTCAATTCCCGTCGTTCGCCC	A
GGCCAATCATTGTAGGTATAATGGTAGATGCTCTGGACCAAGTTATTATATATCTTTTCCGCTTTTGT	c.
3250	•
3250	
1400 1450	
TTAAGTTTATTTATTTTCTTAATAAATGATTCGCTACAAAAGGATTTTTTTT	ã.
TTAAGTTTATTTATTTTTCTTAATAAATGATTCGCTACAAAAGGATTTTTTTT	A
3300 3350	
(74	
1500	-
TTACTCCTTTTTCTTGTAAAGACGAAGAAACAATTTCTATTTTCTCTACTACTATTTAGTACGACGACGAAGAATC	A
TTACTCCTTTTTCTTGTAAAGACGAAGAAACAATTTCTATTTTCTCTACTACTATTTAGTACGACGACGAAGAATC	X
3400	-
1550 1609	_
AATTATCACTATATTTCTTCCTTTTTCTACTTCTTCCAAGTGCAGGAAAACCCCCAAGGAGTTGCGGGTTT	Ŧ
	_
AATTATCACTATATTTCTTCCTTTTTCTACTTCTTCCTAGTGCAGGAAAACCCCCAAGGAGTTGCGGGTTT	T
3450 3500	

TTTCTACCAATTGGGGGCC

TTTCTACCAATTGGGGGCC

<u>Fig. 3</u>. Nucleotide sequence of the <u>trn</u> H gene region, its flanking parts and parts of the inverted repeats IR1 and IR2. Only the RNA like strand is shown. The structural part of the <u>trn</u> H gene and IR1 and IR2 are framed. Note that the <u>psb</u> A and <u>trn</u> H gene have the same polarity. Counting of nucleotide positions is continuous from Fig. 2, note the overlap. For IR1 the strand with the polarity of the <u>trn</u> H gene is given. For IR2 the sequence of the opposite strand (by definition) is given including 70 positions of the large single copy region adjacent to IR2.

in Fig. 3 are counted as in Fig. 2, note the overlap). Within this segment we found the gene for tRNA^{His} ($\underline{\text{trn}}$ H). The structural part and therefore the secondary structure of the transcrift (Fig. 4) are to 100% identical with the recently sequenced $\underline{\text{trn}}$ H gene of Zea mays (16). Upstream of the structural part at positions 1193 to 1198 and 1215 to 1219 we recognize sequences which may qualify as promoter sequences (15). Downstream and already within the IR1 we recognize inverted repeats (9-mer) which could form a stem and loop structure and qualify as gene terminators.

Seventy positions of both ends of the large single copy region and 250



Fig. 4. Cloverleaf structure (unmodified) of soybean chloroplast tRNAHis as deduced from the <u>trn</u> H sequence. The 5' terminal G is taken as position 1. Arrows point towards nucleotides present at that position in <u>Euglena</u> <u>gracilis</u> tRNAHIS (22); del : deletion.

positions of the IR1 and IR2 are aligned and compared in Fig. 3. IR1 and IR2 show perfect sequence homology for the entire analysed segment (250 positions). The two ends of the single copy region have nothing in common within the analysed stretch (70 positions), i.e., the structural part of the <u>trn</u> H gene is certainly not duplicated in this genome contrary to the situation in <u>Zea</u> <u>mays</u> (16). The size and function of an open reading frame which starts at position 3348 within IR2 (Fig. 3) and continues in the large single copy region (coding strand) is presently under investigation.

DISCUSSION

The psb A gene

The soybean chloroplast <u>psb</u> A gene is in its structural properties essentially identical to that of <u>S</u>. <u>oleracea</u> and <u>N</u>. <u>debneyi</u> (6). The length of the transcribed region (distance between the most likely transcription initiation and termination) is well within 1.2 kb, what agrees with the size of the major RNA which interacted with a <u>psb</u> A gene probe (17). These authors compared the <u>psb</u> A gene transcripts of several angiosperm chloroplasts including soybean and spinach. They observed for soybean, but not for spinach, that two minor transcription products of about 1.0 and 0.2 kb also interacted with the <u>psb</u> A DNA probe. They thought, however, that these minor RNAs were specific degradation products of the 1.2 kb RNA, the specific cleavage site being on the 3' terminal part of the coding region. A sequence comparison between the soybean and spinach <u>psb</u> A gene around nucleotide position 900 (Fig. 2), where preferential cleavage would occur in case of soybean, reveals no particular differences, i.e., the soybean chloroplast must contain a ribonuclease(s) which differ in specificity from that of spinach chloroplasts.

Hoffman-Falk et al. (18) studied the Mr 32 000 thylakoid membrane protein from several angiosperms and the alga Chlamydomonas rheinhardii. They found extensive similarities at levels of precursor maturation, membrane orientation and primary structure. This is in accordance with the psb A sequence data published sofar. There is no doubt that constraints on the primary structure of the entire translated region are very rigid, permitting only point mutations (with few exceptions) in the wobble position as shown now in chloroplast genomes of representatives from three distant plant families (Chenopodiaceae, Solanaceae, Leguminosae). Of particular importance is the observation that also in case of soybean the psb A gene codes for 353 aminoacids, the N-terminal part being to 100% homologous with that of S. oleracea and N. debneyi. This strongly suggests that the first 36 codons of the open reading frame are translated and essential in the primary translation product. However, McIntosh (19) reported very recently that the psb A genes from Zea mays and Amaranthus hybridus encode a protein of 317 aminoacids only. According to this preliminary report, it seems possible that the first 36 aminoacids are not required for a functional 32 kd thylakoid membrane protein. More analytical data, however, are necessary to obtain a clear picture concerning the size difference between the coding region and the gene product. Zurawsky et al. (6) discussed several possibilities to explain this discrepancy.

The trn H gene and the terminal part of the inverted repeats

A <u>trn</u> H gene was mapped adjacent to one of the inverted repeats on the chloroplast genomes of <u>Spinacia oleracea</u> (10) and <u>Phaseolus vulgaris</u> (20). For <u>Zea mays</u> the <u>trn</u> H gene was mapped within the inverted repeats and it was shown that the <u>trn</u> H gene slightly overlaps with a gene of opposite polarity which is transcribed into a RNA of 1.6 kb (16). The soybean <u>trn</u> H gene sits with its structural part right at one end of the large single copy region and a potential transcription termination site is located on the inverted repeat. The ribonucleotide sequence deduced from the <u>trn</u> H gene is to 100% identical with that of <u>Zea mays</u> chloroplasts. All highly conserved positions (21) are maintained in the <u>trn</u> H sequence. In Fig. 4 we compare the soybean with the <u>Euglena gracilis</u> chloroplast tRNAHis (22) as deduced from the corresponding structural genes. There is about 80% sequence homology. In both cases the D-stem is shortened and the extra loops are of identical length. The 3' terminal CCA are not encoded in the genes, a property chloroplast tRNA genes seem to share with eukaryotic tRNA genes (21). All three chloroplasts \underline{trn} H genes sequenced so far code for tRNA^{His}_{GUG}. According to extensive hybridization experiments using a variety of chloroplast genomes (for references, see 10) no second \underline{trn} H gene was identified, i.e., a possible gene for tRNA^{His}_{AUG} is still undetected. Nevertheless, we can see (Fig. 2) that the codon CAT (CAU) is frequently used in translating both the spinach and soybean Mr 32 00C thylakoid membrane protein, this in agreement with the wobble hypothesis.

The three chloroplasts \underline{trn} H genes sequenced so far have a different genetic environment. The soybean \underline{trn} H gene is at one end of the large single copy region proximate to and with the same polarity as the <u>psb</u> A gene. Its transcription termination site is most likely part of the inverted repeat. The maize \underline{trn} H gene is integral part of the inverted repeat, occurs therefore twice per circular genome and it overlaps slightly with a protein coding gene of opposite polarity (16). Finally the <u>Euglena gracilis</u> \underline{trn} H gene is the second gene in a cluster of five tandemly arranged tRNA genes (22), which most likely are co-transcribed and under the control of one promoter. \underline{trn} H gene transcription regulation must be different in the three types of chloroplasts, a point of considerable interest.

It is known from cross hybridization experiments that the inverted repeats of chloroplast genomes are structurally related, displaying considerable sequence homology (7). Our sequence results define for the first time the exact beginning of IR1 and IR2 on a angiosperm chloroplast genome. A comparison with the Zea mays chloroplast IR (16) reveals that a short stretch of 32 positions is to 91% homologous with a soybean chloroplast DNA segment (position 1510-1542). Most likely, sequence homology between the two IRs continues beyond the sequenced positions (16). It's noteworthy that the short homologous parts map at different places within the two kinds of inverted repeats. In case of soybean this short segment starts 138 positions inside of the beginning of the IR, for maize it is most likely about 0.5 kb away from the IR start. This and the different location of the \underline{trn} H gene shows that during evolution not only the single copy region underwent DNA rearrangments but also the rather conservative inverted repeats region.

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