Title

THE IDENTIFICATION, ISOLATION, AND SEQUENCE OF THE REACTION CENTER PROTEIN GENES OF THE PHOTOSYNTHETIC PURPLE BACTERIUM RHODOPSEUDOMONAS CAPSULATA

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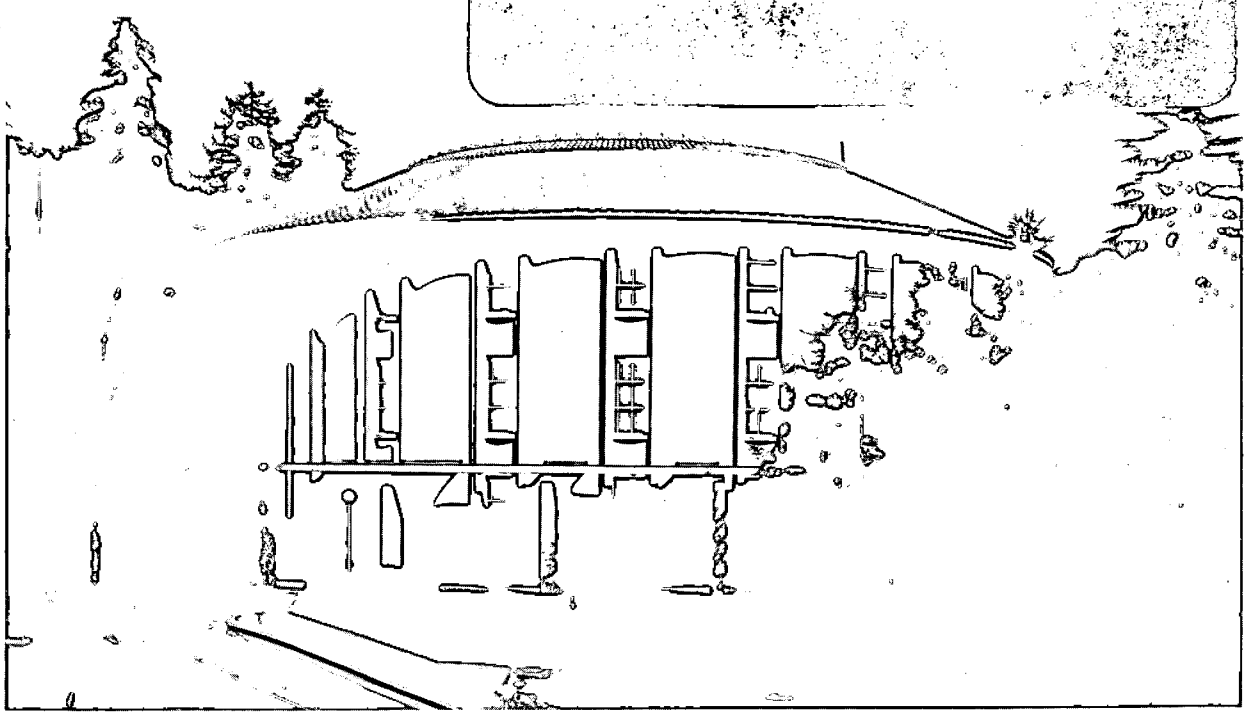

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DISCLAIMER

THE IDENTIFICATION, ISOLATION, AND SEQUENCE OF THE REACTION CENTER PROTEIN GENES OF THE PHOTOSYNTHETIC PURPLE BACTERIUM RHODOPSEUDOMONAS CAPSULATA

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ABSTRACT

Reaction centers in photosynthetic membranes are the centers to which electronic excitation due to light absorption is transferred. This excitation brings about a charge separation between a bacteriochlorophyll molecule and two quinone molecules which ultimately leads to the formation of a hydroquinone. The reduced hydroquinone is then utilized to produce a proton gradient across the membrane and ultimately to produce ATP. We have focused our interest on the structure of the reaction center in the photosynthetic purple bacterium, Rhodopseudomonas capsulata, with the intention of establishing a detailed understanding of these first chemical steps in the natural fixation of sunlight. In this manuscript, I outline the methods that I and my coworkers have used to identify and isolate the genes for the three reaction center subunits, L, M, and H, in Rps. capsulata.

These genes have then been sequenced, and the sequences analyzed in detail for their similarity with sequences of comparable proteins from more advanced photosynthetic bacteria such as Anabena, from algae such as Euglena and Chlamydomonas, and from higher plants such as amaranthus, soybean, tobacco and spinach. Homology was found which has been tentatively interpreted to be in the region of quinone binding in all of these reaction centers. There is growing optimism that there will be sustantial structural similarity between the reaction centers of the purple bacteria and those of photosystem II in higher plants. This conclusion is important because the X-ray crystal structures of several of the purple bacteria reaction center complexes are presently being worked on and will ultimately be solved.

TEXT

We initiated a research program in the Melvin Calvin Laboratory at the University of California on the molecular genetics of photosynthesis about three years ago. We selected Rps. capsulata as the organism of choice entirely because of the prior activity of Professor Barry Marrs whose laboratory had developed gene transfer systems for this organism and had completed the mapping of several bacteriochlorophyll biosynthesis genes and carotenoid biosynthesis genes related to photosynthesis (Yen and Marrs, 1976). Marrs (1981) had isolated a plasmid, a derivative of an R factor, RP1, which grew in E. coli, could be conjugated into Rps. capsulata, and which contained a Rps. capsulata gene cluster capable of complementing all photosynthesis mutants that had been isolated at that time. This plasmid is called pRPS404, contains 116 kilobasepairs of DNA of which the 45 kb section contained between the IS 21 elements or between map positions 23 and 68 in Figure 1 is the Rps. capsulata photosynthesis gene cluster. Figure 1 contains a restriction map worked out by the joint activities of the Marrs laboratory and this laboratory.

Next we developed procedures for the transposon mutagenesis of pRPS404 in E. coli which allow for easy mapping of the positions of the transposon insertions (Youvan et al. 1982). In order to improve the stability of the mutants generated in this way, we created a new transposon called Tn5.7 which has the transposition characteristics of Tn5 and the antibiotic resistance characteristics of Tn7 (Zsebo, Wu and Hearst (1984). Upon conjugation of the mutated pRPS404 into Rps. capsulata, site directed mutation occurs at the same map position in the Rps. capsulata genome as it occurred in the E. coli clone from which it was derived. Recombination is not accompanied by complex DNA rearrangements or deletions (Zsebo and Hearst, 1984). By screening both the

earlier mutants generated by the Marrs laboratory and the transposon mutant generated in this laboratory for fluorescence in the infra-red, we were able to identify two map positions separated by 35 kb which were prime candidates for DNA sequencing. They contained the sequences of the reaction center proteins (Youvan, Hearst and Marrs, 1983).

Youvan et al. (1984b) have just reported the primary sequence of these two 4000+ basepair fragments of Rps. capsulata which code for the light harvesting I polypeptides, B870α and B870β, the three reaction center polypeptides H, M, and L, and for some presumed regulatory functions relating to the synthesis of these components of the photosynthetic membrane (Zsebo and Hearst, 1984). From the deduced amino acid sequences of the reaction center subunits it was concluded that the H subunit is a hydrophilic protein with a very hydrophobic amino terminus of thirty amino acids. The H subunit is a polypeptide 254 amino acids long with a molecular weight of 28,534 daltons. The L and M subunits are both very hydrophobic proteins with 282 and 307 amino acids respectively. Their molecular weights calculate to be 31,565 and 34,440 daltons, indicating that they have anomolous SDS PAGE electophoretic mobilities because of their hydrophobic characters. Hydropathy plots suggest that the L and M subunits are transmembrane proteins which may cross the membrane five times.

Feher and Okamura (1978) have shown that the H subunit is not essential to the electron transfer activities of the reaction centers in Rps. sphaeroides. While the H, M, and L subunits always occur in equimolar quantities in reaction centers, the H subunit is thirty-five kilobasepairs away from the operon containing the LH B870α, LH B870β, L subunit, and M subunit in Rps. capsulata (Youvan et al., 1984a) and so the H subunit gene is clearly under separate transcriptional control from the other structural proteins in the reaction center. Chory et al. (1984) have suggested that the

H subunit serves as the structural anchor in the membrane around which the reaction center forms and evidence is presented suggesting that the H subunit can be found in membrane fractions isolated even from aerobically grown cells.

We are therefore at a point where it is generally accepted that the only two polypeptides essential for reaction center activity are the L and M subunits in Rps. sphaeroides. These two subunits must contain binding sites for the strongly bound and weakly bound ubiquinones, Q_A and Q_B, for the Fe(II), and for the bacteriochlorophylls and the bacteriopheophytins. Of these interactions, I consider the sites for binding the quinones most crucial since they serve the roles of preserving or stabilizing very labile anion radicals which are one electron intermediates in the quinone to hydroquinone reduction pathway. This model supposes that pigment binding functions are more variable and therefore less tightly conserved in evolution.

Hearst and Sauer (1984a, 1984b) have discovered that there is a highly conserved pattern of sequences of amino acids which is common to the L and M subunits of Rps. capsulata and the Q_B-protein of chloroplast thylakoid membranes in spinach and tobacco. The conservation has survived an estimated three billion years of evolution (J. M. Olson, 1981). Such a striking conservation of amino acid sequence suggests that these portions of all three proteins, all of which are approximately 60% in from the amino terminus, are at the functional centers of these proteins. It is their hypothesis that these highly conserved sequences of amino acids are involved in quinone binding and function.

There is a large literature of physical measurements on thylakoid membranes from higher plants which establish the functional and compositional similarities between photosystem II reaction centers in these membranes and

the reaction centers of Rps. capsulata and Rps. sphaeroides. The sequences of the corresponding proteins in plants and purple bacteria now have proven the evolutionary relationships between these reaction center proteins. I would like to suggest here that all PS II reaction centers are likely to contain two similar protein subunits with molecular weights between 30 kd and 40 kd with roles analogous to those of the L and M subunits in the reaction centers of photosynthetic purple bacteria. I am stressing quinone binding here although common features of pigment binding might also prevail. The strongest evidence in favor of this position comes from the recent nucleic acid sequence data of J.-D. Rochaix et al. (1984) and of O.F. Rasmussen et al. (1984). Rochaix et al. (1984) present the nucleic acid sequence of the psbD locus of the Chlamydomonas reinhardii chloroplast genome. The psbD gene codes for the D2 polypeptide which has been associated with photosystem II (Chua and Gillham, 1977) together with its partner, the D1 polypeptide, which is coded by the psbA gene and which corresponds to the 32 kd herbicide binding protein or Q_B protein of higher plants. Rochaix et al. make the striking observation that while the overall homology between the Q_B protein and the D2 protein of C. reinhardii is only 27%, there are several domains where one can find homology ranging between 33% and 58%. Q_B contains 352 amino acids while D1 contains 339 or possibly 350 amino acids. The putative quinone binding region is highly conserved in both proteins, with Q_B corresponding best with the L subunit of Rps. capsulata and D2 corresponding best with the M subunit of Rps. capsulata.

Rasmussen et al. (1984) have sequenced the corresponding psbD gene from the higher plant chloroplast genome, Pisum sativum or pea. Their D2 protein is 353 amino acids long and shows an 84.7% homology to the D2 protein of C. reinhardii. Again the putative quinone binding region is the most highly

conserved region of the protein relative to the M subunit of Rps. capsulata.

Figure 2 shows an alignment of the amino acid sequences of the L subunit of Rps. capsulata (Youvan et al., 1984b) with the Q_B proteins of spinach and tobacco (Zurawski et al., 1981), of soybean (Spielmann and Stutz, 1983), of Amaranthus hybridus (Hirschberg and McIntosh, 1983), of C. reinhardii (Erickson et al., 1983), of Euglena (Hallick, 1984), and of Anabena I (Hazelkorn, 1984). Overall, there is 21.5% homology between the L subunit and the Q_B protein of spinach, 21.0% homology between the L subunit and the Q_B protein of C. reinhardii and 92.3% homology between the two Q_B proteins. The most striking feature of this figure is the strong homology between all seven proteins in the hypothetical quinone binding region: N----PFHMLG----F------AMHG-LV-S.

Figure 3 shows an alignment of the amino acid sequences of the M subunit of Rps. capsulata (Youvan et al., 1984b), the D2 protein of pea (Rasmussen et al., 1984) and the D2 protein of C. reinhardii (Rochaix et al., 1984). Overall, there is 20.8% homology between the M subunit and the D2 protein of pea, 19.5% homology between the M subunit and the D2 protein of C. reinhardii and an 84.7% homology between the two D2 proteins. The most striking feature of this figure is the strong homology between all three proteins in the hypothetical quinone binding region: F---H-N---NPFH----A---G-ALL-A-HGAT.

In conclusion, I am suggesting that the psbA and psbD loci of chloroplast genomes encode for the photosystem II analogs of the L and M reaction center subunits of R. capsulata and other photosynthetic purple bacteria. While the corresponding chloroplast proteins, D1 and D2, appear to have molecular weights of 32 to 34 kd by SDS PAGE, they are very hydrophobic membrane bound proteins which may cross the membrane five or more times. Their true molecular weights will be between 38 and 40 kd (Zurawski et al.,

1981 and Rasmussen et al., 1984). Hearst and Sauer, 1984a and 1984b have proposed that the most highly co-conserved sequences between the L subunit, the M subunit and the Q_B protein are involved in quinone binding. While Wolber and Steinback (1984) have proven that the trypic peptide of the Q_B protein which contains the presumptive quinone binding site also contains the major site for the covalent linkage between azidoatrazine and the herbicide binding protein in thylakoid membranes, direct physical evidence relating to quinone binding remains elusive and requires further experimentation. In this spirit, it must be remembered that identification of the Q_B protein with the weak quinone binding site, and the D2 protein with the strong binding site relates to a model and cannot yet be considered experimental fact.

ACKNOWLEDGEMENTS

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BIBLIOGRAPHY

Chory, J., Donohue, T.J., Varga, A.R., Staehelin, L.A., Kalplan, S. (1984). Reduction of the Photosynthetic Membranes of Rhodopseudomonas sphaeroides: Biochemical and Morphological Studies. In press.

Chua, N.H., Gillham, N.W. (1977). The sites of synthesis of the principal thylakoid membrane polypeptides in Chlamydomonas reinhardii. J. Cell Biol. 74, 441-452.

Erickson, J.M., Schneider, M., Vallet, J.M., Dron, M., Bennoun, P., Rochaix, J.D. (1983). Chloroplast gene function: combined genetic and molecular approach in Chlamydomonas reinhardii. Proceedings of 6th International Congress on Photosynthesis, Sybesma, C., editor, M. Nijhoff, W. Junk Publ., in press.

Feher, G., Okamura, M.Y. (1978). Chemical composition and properties of reaction centers. The Photosynthetic Bacteria (Clayton, R.K., and W.R. Sistrom, eds.), pp. 349-386.

Hallick, R. (1984). In press.

Hazelcorn, . (1984). In press.

Hearst, J.E., Sauer, K. (1984a). Protein sequence homologies between portions of the L and M subunits of reaction centers of Rhodopseudomonas capsu-

lata and the 32 kd herbicide-binding polypeptide of chloroplast thylakoid membranes and a proposed relation to quinone-binding sites. In Advances in Photosynthesis Research, Proceedings of the VIth International Congress of Photosynthesis, C. Sybesma, ed., Vol. III.5, 355-359.

Hearst, J.E., Sauer, K. (1984b). Protein sequence homologies between portions of the L and M subunits of reaction centers of Rhodopseudomonas capsulata and the Q_B-protein of chloroplast thylakoid membranes: A proposed relation to quinone-binding sites. Z. Naturforsch., in press.

Hirschberg, J., McIntosh, L. (1983). Molecular basis of herbicide resistance on Amaranthus hybridus. Science 222, 1346-1349.

Marrs, B. (1981). Mobilization of the genes for photosynthesis from Rhodopseudomonas capsulata by a promiscuous plasmid. J. Bacteriol. 146, 1003-1012.

Olson, J.M. (1981). Evolution of photosynthetic and respiratory prokaryotes and organelles. In Fredrick, J.F., ed. Origins and Evolution of Eukaryotic Intracellular Organelles, pp. 8-19. New York Academy of Sciences.

Rasmussen, O.F., Bookjans, G., Stummann, B.M., Henningsen, K.W. (1984). Localization and nucleotide sequence of the gene for the membrane polypeptide from pea chloroplast DNA. Plant Mol. Biol., submitted.

Rochaix, J.-D., Drom, M., Rahire, M., Malnoe, P. (1984). Sequence homology

between the 32 kd and the D2 chloroplast membrane polypeptide. Plant Mol. Biol., submitted.

Spielmann, A., Stutz, E. (1983). Nucleotide sequence of soybean chloroplast DNA regions with contain the psb A amd trn H genes and cover the ends of the large single copy region and one end of the inverted repeats. Nucleic Acids Research 11, 7157-7167.

Wolber, P.K., Steinback, K.E. (1984). Identification of the herbicide binding region of the Q_B-protein by photoaffinity labeling with azidoatrazine. Z. Naturforsh., in press.

Yen, H.-C. and B. Marrs (1976). Map of genes for carotenoid and bacteriophyll biosynthesis in Rhodopseudomonas capsulata. J. Bacteriol. 126, 619-629.

Youvan, D.C., Elder, J.T., Sandlin, D.E., Zsebo, K., Alder, D.P., Panopoulos, N.J., Marrs, B.L. and Hearst, J.E. (1982). R-Prime site-directed transposon Tn7 mutagenesis of the photosynthetic appartus in Rhodopseudomonas capsulata. J. Mol. Biol. 162, 17-41.

Youvan, D.C., Hearst, J.E. and Marrs, B.L. (1983). Isolation and characterization of enhanced fluorescence mutants of Rhodopseudomonas capsulata. J. Bacteriology 154, 748.

Youvan, D.C., Alberti, M., Begusch, H., Bylina, E.J., Hearst, J.E. (1984a). Reaction center and light harvesting I genes from Rhodopseudomonas

capsulata. Proc. Nat. Acad. Sci. USA 81, 189-192.

Youvan, D.C., Bylina, E.J., Alberti, M., Begusch, H., Hearst, J.E. (1984b). Nucleotide and deduced polypeptide sequences of the photosynthetic reaction center, B870 antennae and flanking polypeptides from Rps. capsulata. Cell, in press.

Zsebo, K.M. and Hearst, J.E. (1984). Genetic-physical mapping of a photosynthetic gene cluster from Rhodopseudomonas capsulata. Cell, in press.

Zsebo, K.M., Wu, F. and Hearst, J.E. (1984). Tn5.7 construction and physical mapping of pRPS404 containing photosynthetic genes from Rhodopseudomonas capsulata. Plasmid 11, 182-184.

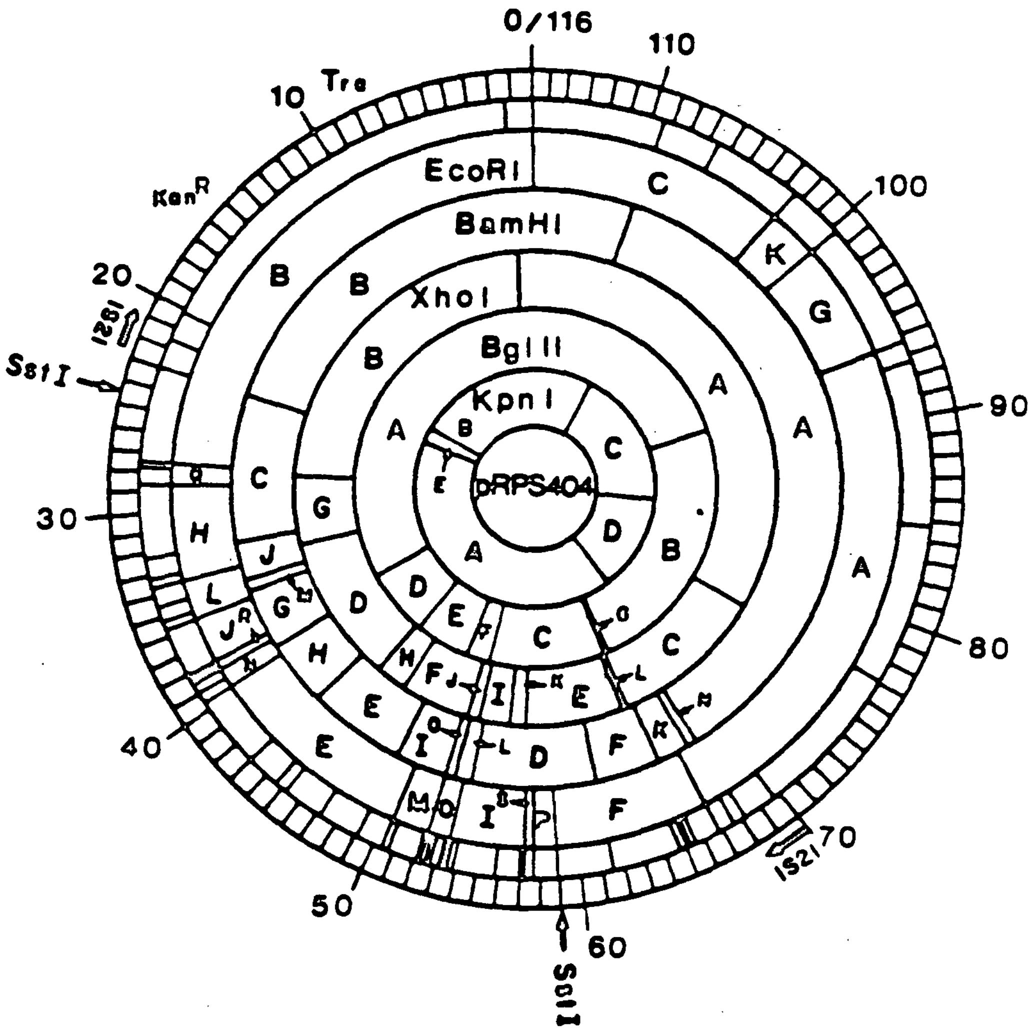
Zurawski, G., Bohnert, H.J., Whitfield, P.R., Bottomley, W. (1981). Nucleotide sequence of the gene for the Mr 32,000 thylakoid membrane protein from Spinacia oleracea and Nicotiana debneyi predicts a totally conserved primary translation product of Mr 38,950. Proc. Nat. Acad. Sci. USA 79, 7699-7703.

FIGURE LEGENDS

Figure 1. Physical map of pRPS404. Arrows indicate IS21 elements which bracket the Rps. capsulata DNA. The Tn5.7 insertions generated during the transposon mutagenesis studies are named for their positions in kilobases on this map.

Figure 2. An amino acid sequence alignment for the L subunit reaction center polypeptide of Rps. capsulata, the herbicide binding thylakoid membrane protein, Q_B, of spinach and tobacco, of soybean, of Amaranthus hybridus, of C. reinhardii, of Euglena, and of Anabena. This figure was created with the kind assistance of Professor Richard Hallick of the University of Colorado in Boulder.

Figure 3. An amino acid sequence alignment for the M subunit reaction center polypeptide of Rps. capsulata, the D2 protein of pea, and the D2 protein of C. reinhardii. The two D2 proteins are encoded by the psbD loci of the chloroplast genomes of their respective organisms. There are 64 matches and 20.8% homology between the M and D2 pea, 60 matches and 19.5% homology between the M and D2 chlamydomonas, and 287 matches and 84.7% homology between D2 chlamydomonas and D2 pea.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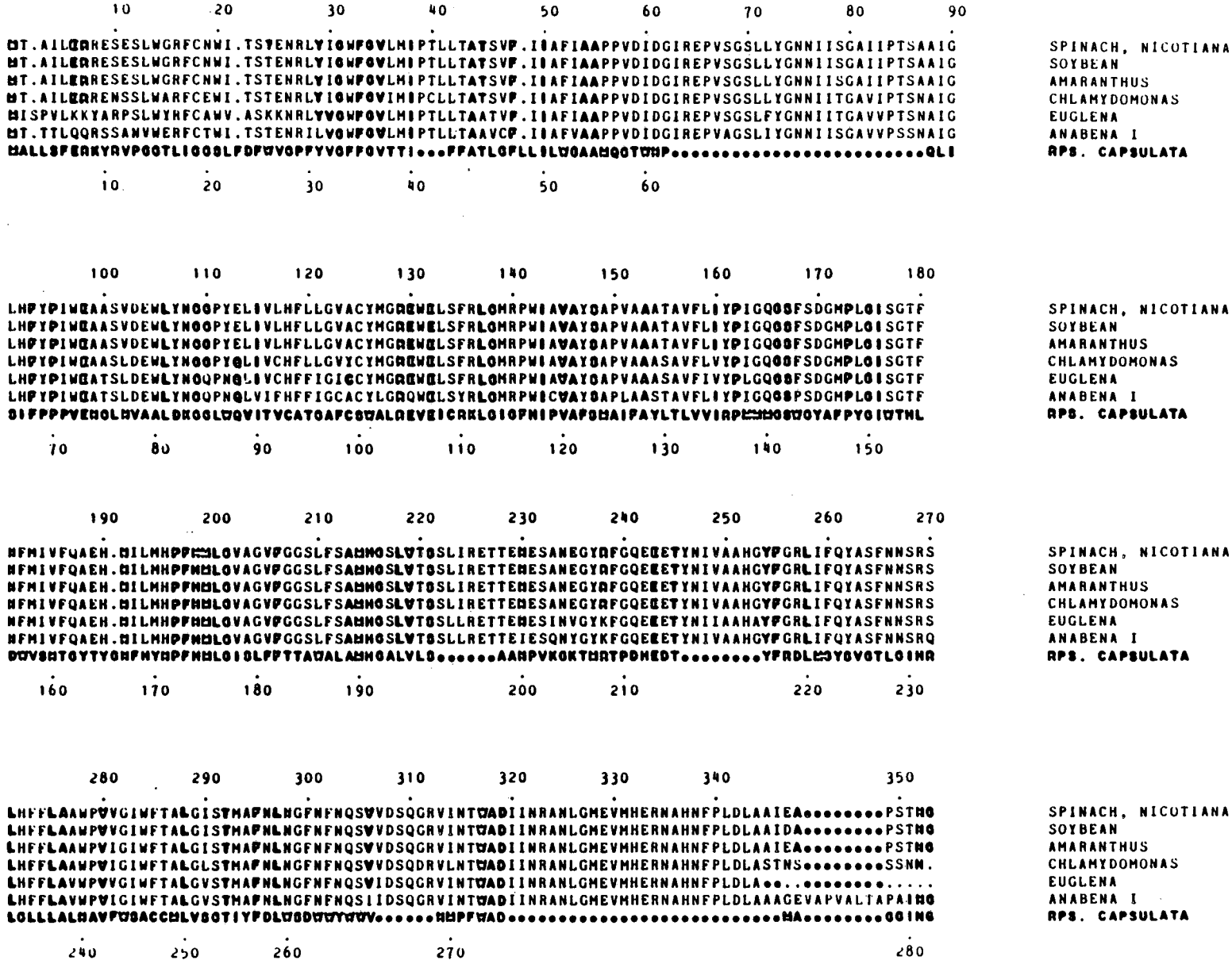
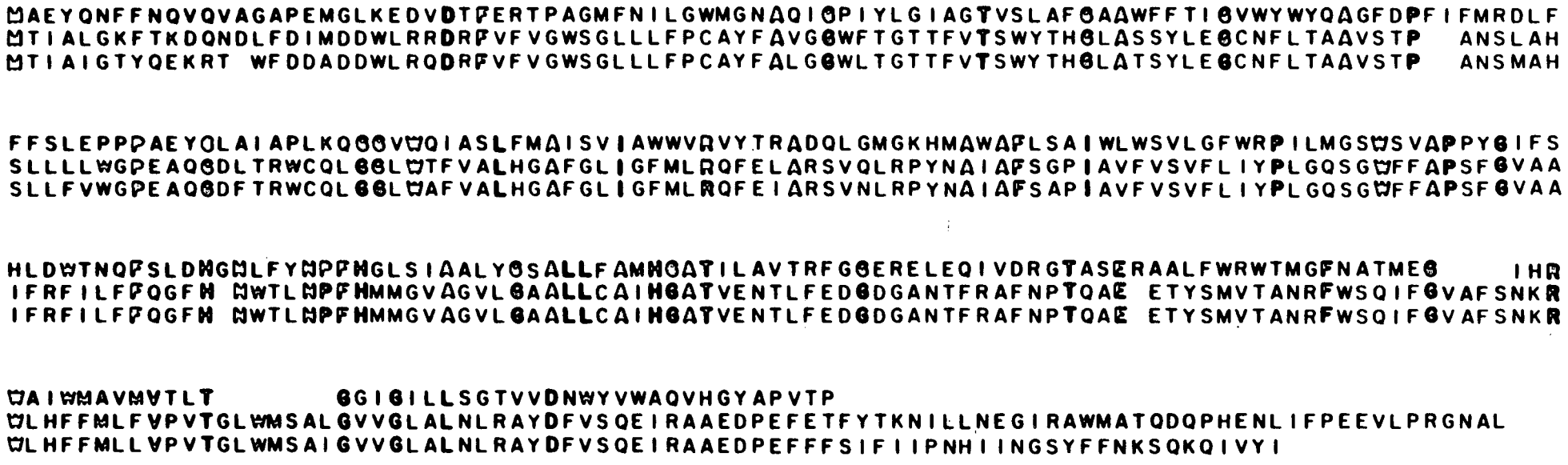
IS21
SstI
SalI
EcoRI
BamHI
XhoI
BglII
KpnI
pRPS404

Figure 1



SPINACH, NICOTIANA	(353)	: RPS. CAPSULATA	76 (21.5%)	: SPINACH	353 (100%)
SOYBEAN	(353)	: RPS. CAPSULATA	75 (21.2%)	: SPINACH	352 (99.7%)
AMARANTHUS	(353)	: RPS. CAPSULATA	75 (21.2%)	: SPINACH	351 (99.4%)
CHLAMYDOMONAS, psbA	(352)	: RPS. CAPSULATA	74 (21.0%)	: SPINACH	325 (92.3%)
EUGLENA	(345)	: RPS. CAPSULATA	62 (18.0%)	: SPINACH	305 (86.4%)
ANABENA I	(361)	: RPS. CAPSULATA	70 (19.4%)	: SPINACH	296 (83.9%)
RPS. CAPSULATA	(282)	: RPS. CAPSULATA	282 (100%)	: SPINACH	76 (21.5%)

Figure 2



Amino Acid Sequence Alignments for :

M Subunit	*Rhodopseudomonas capsulata*	(307 amino acids)
D2 Protein (psbD)	*Pisum sativum*	(353 amino acids)
D2 Protein (psbD)	*Chlamydomonas reinhardii*	(352 amino acids)

Figure 3

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