MINI-REVIEW

Amino Acid Identities in the Three Redox Center-Carrying Polypeptides of Cytochrome $bc_1/b_6 f$ Complexes

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Abstract

The comparison of primary structures is extended to 22 cytochromes b or b_6 , 12 cytochromes c_1 or f, and 8 Rieske FeS proteins. Conclusions are drawn as to their phylogenetic relationship as well as on conserved, functionally important amino acids and secondary structures. The results are in favor of two independent quinone binding sites at opposite surfaces of the membrane, topping one of the two hemes of cytochrome b each.

Key Words: Cytochrome bc_1 complex; cytochrome $b_{\delta}f$ complex; Rieske FeS protein; amino acid sequences; quinone-binding peptides; membrane proteins; organelle gene evolution.

Introduction

Cyt $bc_1/b_6 f$ complexes function as quinol-cyt c or plastocyanin oxidoreductases in many respiratory and all photosynthetic electron transport chains so far studied (Hauska *et al.*, 1983; Hauska, 1985, 1986a). Active $b_6 f$ complexes from chloroplasts and cyanobacteria, and bc_1 complexes from photosynthetic bacteria, can be isolated by a general procedure (Hauska, 1986b), which has

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been improved recently (Ljungdahl *et al.*, 1986). These complexes are remarkably simpler than their mitochondrial counterparts (Schägger *et al.*, 1986). Essentially only three polypeptides are found, which represent cyt f or c_1 , cyt b, and the Rieske FeS protein (Hauska *et al.*, 1983; Hauska, 1985, 1986a). In the $b_6 f$ complexes the cyt b component (ca. 42 kD) is split into cyt b_6 (23 kD) and subunit IV (15.2 kD) (Heinemeyer *et al.*, 1984). In addition to electron transport from quinol to cyt c or plastocyanin, these complexes translocate protons charging the membrane. The mechanism of this reaction is complex, and different concepts—Q cycle, b cycle, semiquinone cycle—are discussed (Hauska *et al.*, 1983; Crofts, 1985). Recently, a whole issue of *J. Bioenerg. Biomembr.* (No. 3, 1986) has been devoted to this matter.

Earlier studies on the accessibility for antibodies, and analyses of the primary structures, led to a universal topographical model for the $bc_1/b_6 f$ complexes (Hauska *et al.*, 1985; Hauska, 1985, 1986a; see Gabellini and Link *et al.*, in Colson, 1987). It is especially detailed for the cyt *b* component, because several sequences were compared, and because hydrophobic spans suggest that it folds nine times through the membrane (Saraste, 1984; Widger *et al.*, 1984; see Crofts in Colson, 1987). This model is in striking agreement with current functional concepts.

Recently this study has been extended from six (Saraste, 1984; Widger *et al.*, 1984) to eleven different cyt b/b_6 sequences, with the aim of predicting functional centers, especially quinone binding sites, and to find indications for the different inhibitor sensitivities of bc_1 and $b_6 f$ complexes (Nitschke and Hauska, 1987a; Lang *et al.*, 1985; Schuster and Brennicke, 1985). The sequence of cyt *b* from *Trypanosoma* (Benne *et al.*, 1983) was of particular importance in pinpointing essential residues. It shows only about 20% identity to any of the other sequences, but the functional characteristics, like antimycin sensitivity (Markos *et al.*, 1986), seem to be retained.

Here we extend the sequence comparison to 22 cyt *b* sequences. In addition we compare 12 cytochromes c_1 or *f*, and 8 Rieske FeS proteins. The sequences are aligned for maximal identity, without penalties for deletions. This might somewhat distort evolutionary distances. We hope that besides its contribution to our understanding of the function and evolution of the $bc_1/b_6 f$ complexes, such a study will be of value for directed mutagenesis.

Cytochrome $b = b_6 +$ subunit IV

Cytochrome *b* leads membrane proteins with respect to the number of elucidated primary structures (Fig. 1). By now these are known for 14 mitochondria—man = H (Anderson *et al.*, 1981a), beef = B (Anderson

et al., 1981b), mouse = M (Bibb *et al.*, 1981), rat = R (Koike *et al.*, 1982), toad = T (Roe *et al.*, 1985), the protozoa *Trypanosoma brucei* = Tr (Benne et al., 1983) and Leishmania tarantolae (de la Cruz et al., 1984; not shown), the yeasts Saccharomyces cerevisiae = Y (Nobrega and Tzagoloff, 1980) and Schizosaccharomyces pombe (Lang et al., 1985; not shown), the fungi Neurospora crassa = Nc (Fig. 1 shows the partial sequence of Burke et al., 1984, because of higher identity, completed by the data from Helmer-Citterich et al., 1983) and Aspergillus nidulans = A (Waring et al., 1981), and the plants maize = Mz (Dawson et al., 1984), wheat = Wh (Boer et al., 1985), and Oenothera = Oe (Schuster and Brennicke, 1985). Furthermore, Fig. 1 shows the sequences of cytochromes b for three bacteria-Paracoccus denitrificans = Pc (Kurowski and Ludwig, 1987) and two strains of *Rhodobacter capsulatus* = Rc1, Rc2 (Gabellini and Sebald, 1986; Davidson and Daldal, 1987a); originally Rc1 was considered to be Rhodopseudomonas sphaeroides GA (Gabellini and Sebald, 1986), but later was identified as R. capsulatus (Davidson and Daldal, 1987b; see also Gabellini, 1988)-and for three chloroplasts $(b_6 + \text{subunit IV})$ —spinach = Sp (Heinemeyer et al., 1984), tobacco = Tb (Shinozaki et al., 1986), and liverwort = Lw (Ohyama et al., 1986). In addition, a partial sequence for b_6 + subunit IV of the cyanobacterium Nostoc (Kallas et al., 1986; R. Malkin, personal communication; not shown) and the sequence of subunit IV from pea (Phillips and Gray, 1984) are known.

In Fig. 1, 19 of these sequences are aligned in blocks of 75 amino acids in single-letter code. Mistakes found in earlier alignments (eight in Saraste, 1984 and two in Widger et al., 1984) have been corrected for the original publications. The N-terminal sequences of spinach b_6 and subunit IV have been changed from previous presentations (Heinemeyer et al., 1984; Widger et al., 1984; Nitschke and Hauska, 1987a) after the discovery of introns (Shinozaki et al., 1986; Ohyama et al., 1986; Westhoff and Herrmann, unpublished). This correction has been confirmed for spinach by amino acid sequencing (Pfefferkorn and Meyer, 1987), the initial M being cleaved off in b_6 as well as in subunit IV. Pair identities and nonconservation exchanges are indicated by vertical and horizontal lines, respectively, leaving conservative exchanges within the groups QNED, RKH, VLIMF, GAP, FYW, ST, and C (Dayhoff, 1978) unmarked. Complete identity (large dots) and complete conservation of related residues (small dots), including *Trypanosoma*, is shown at the bottom, and that excluding Trypanosoma is shown between Rc₂ and Lw. For the bc_1 complexes only, the same is shown at the top and between Y and Nc. Identity of the b_6 /subunit IV sequence is about 90% (Table I) and useless to mark. The nine hydrophobic spans, probably representing transmembrane helices (Saraste, 1984; Widger et al., 1984; Nitschke and Hauska, 1987a), are lined on top and bottom, indicated by



Fig. 1. Alignment of cyt b/b_6 + subunit IV (see text for description).

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в:	SNNPT-GISSOV	-DRIP	ғнрұү	-TIKDIIGALLL	ILALMILYLFAPOL	GOPONYTPANP
M:	SNNPTGLNSDA	DKIP	гнруу	-TIRDILGILIM	FLILATIVIPEPON	GDPONYMPANP
R:	SNNPTGLNSDA	DRTP	-FHPYY	-TIKOLLGVFML	LIFUTIVIFFPDL	GDPDNYTPANP
т:	SINPT-GINSDP	DRVP	-FHPYF	-SYKOLIGFLIM	LTALTLI AMPSPNLI	GDPDNFTPANP
Tr:	SDAFCDRFAFYC	-ERIS-	-FOMME	-YTROMFTAFST	LONNYVIETNWYE	THEFSWYTYDT
Y:	SSNPL	-DRTP	MHSYF	-TRVIVIONATI-	MUTALEVEVSPAT	CHENNYTECNE
NC	SSNPL-GVSCNY		-FAPYY	-LEROLITTETE	TVVISSEVERNUNV	COSENVENAND
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NC:	MOTPPAT VPEWYLLPFYAILR				AMESALLAIMILLPL	TUL-GRSKGLOF
A:	MQTPPATYPEWYLLPFYAILE			STENKITCAT	AMPAALLALMVMPI	TDL-SKLRGVOF
Mz:	MPTPPHIVPEWYFLPIHAILE			SIPDKAGGVA	ALAPYFISILALPF	FKE-MYVRSSSF
Wh:	MPTPPHIVPEWYFLPIHAILR			SIPDKAGGYA	ALAPVEISLIALPE	FKE-MYVRSSSF
0e:	MSTPPHIVPEWYFLPIHAILR			SHPDKAGGVA	ALAPVFICLIALPF	FKD-MYVRSSSF
PC:	LVTPAHIVPEWYFLPFYAILE	AL TADVI	AAMTANATZ	I GI I DAKFIGVI	AMEGALLYMALVPW	LDT-SKAK2COX
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Rc2:	ISTPAHIVPEWYFLPFYAILE	AFAADW	WVILVDGLI	TGI VDAKFTÇVI	AMPGALAVMALAPW	LDI-SEVRSCAY
LW:	FATPLEILPEWYFFPVFQILF	2		TVPNKLLGVI	LMAAVPAGLLTVPF	LENVNKFONPFR
Tb:	FATPLEILPEWYFFPVFQILF	{		TVPNKLLGVL	LHVSVPAGILTVPF	LENVIKEONPER
Sp:	FATPLEILPEWYFFPVFQILF	{			l m asypaglitypf	LENVNRFONPER
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в:	RPLSQCLFWALVADLLTLTWI	GCOPVEL	PYITICOL	SVLYFILLUVI	PTAGTIENKLIKW-	
M:	RPITOILYWILVANLLILIWI	CCOPVEL	PFIIIGOLA	SISYFSITLI	PIŚCIIEDKMIKTY	P
R:	RPITOILYWILVANLLVLIWI	GCOPVER	PFIIIGOLA	sisyfsittitt	PISCIVEDRMIKWN	
т:	RPFTOIMFWALVADTLILIWI	GGOPVEI	DPYTMIGOLA	SVIVESIFTIME	PIMGWVFNKLINW-	-
Tr:	LINETYSLILFYSIWMSGFLA	LYVVLAY	PIWELOV	VILLETLIVCET	ñ	
Y:	KVISKFFFFIFVFNFVLLGOI	GACHVE	PYVINGOTA	TFITFATFLIT	EVISTIENVLEVIG	RVNK
Nc:	RPLSKFAFWAFVVNFLTIMKT	GACHVES	PETERCOFS	TEVESVETETV	PVISLIENTINDIA	VT.K
A:	RPLSKVVFYTFVANFT.TLMOT	GAKHVET	PETERCOTS	TTYPAVEEVIV	PVVSLIENTIVELG	TRANE
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Fig. 1. Continued.

roman numbers. The four conserved histidines probably binding the two hemes between helices II and V (Saraste, 1984; Widger *et al.*, 1984; Nitschke and Hauska, 1987a) are marked by arrows. The rather polar span IV, possibly an amphipathic helix, is dashed and might actually not span the membrane. This is favorable in view of inhibitor resistance sites on the cyt *b* gene in yeast mutants (Colson, 1987, and below). If this is true, the heme binding helices II and V would run in parallel and not antiparallel (see Link *et al.* in Colson, 1987) through the membrane. In addition, conserved charges are marked in Fig. 1, and conserved aromatic residues and β -turn tetrapeptides of high probability (Chou and Fasman, 1979) are indicated by asterisks and arcs, respectively—on the top for the bc_1 , on the bottom for the $b_6 f$ complexes.

Besides the hydrophobic spans and the four conserved histidines in helix II and V, which are stabilized by positive charges at the membrane surfaces, possibly interacting with the carboxyethyl groups of the heme rings. the other essentials of the earlier comparisons (Saraste, 1984; Widger et al., 1984; Nitschke and Hauska, 1987a) are also retained in Fig. 1. There are eight conserved glycines six of them within hydrophobic spans. These are the positions 59 and 83 in I, 91 in the β -turn before II, 135 and 149 in III. 176 and 185 in IV, and 347 in VII. Three conserved prolines are found: one in span IV at 173, one in the heme binding helix V at 206, and one in a β -turn at 309. The one in helix V is of particular interest because it has been suggested that *cis/trans*-isomerization of prolines might play a role in the vectorial function of membrane proteins (Brandle and Deber, 1986). Hydroxylated amino acids are conserved at position 103 in the heme binding helix II, and at positions 193, and 303. Conserved aromatic residues are at positions 56, 124, 132, and 150, both in III, and 160, 258, 259, 311, and 312. Besides the helix stabilizing positive charges 95 and 115 at the boundaries of II, 196 at V and 344 at VII, and the negative charge 267 at VII, one additional positive charge at 321 and two negative charges at 87 and 310 are found. A striking region of identity includes the peptides PEWY312 and ILR321, between helix VI and VII. We suggest that this might form part of the quinol oxidizing site Oo (Nitschke and Hauska, 1987a), with E310 stabilizing the positive charge at R321 which might bind the semiquinone anion (Rich, 1984). In addition, we consider YY260 to be involved in the quinone reduction site Qi (Nitschke and Hauska, 1987a), which is sensitive to antimycin in bc_1 complexes. The insensitivity of the $b_6 f$ complexes to this inhibitor could be related to the split of cytochrome b into b_6 and subunit IV in this region. Further support comes from the mapping of diuron resistance at Y259 in yeast (Colson, 1987). A second diuron resistance site is at the aromatic residue 56, and unless long distance interactions are invoked. positions 56 and YY260 should be close to the Qi-binding pocket. Consequently, as mentioned above, previous folding models of cyt *b* need revision, span IV possibly not traversing the membrane (Saraste, 1984; Widger *et al.*, 1984; Nitschke and Hauska, 1987a; see Crofts in Colson, 1987). Still, our observation that all conserved aromats are found close to the two membrane surfaces (Nitschke and Hauska, 1987a) holds, and is in favor of two independent sites Qo and Qi, as pictured in the Q cycle (Hauska *et al.*, 1983; Crofts, 1985). Support for this notion comes also from a mutant of *Rhodobacter capsulatus* with an inactive Qo-site, but an unchanged Qi-site (Robertson *et al.*, 1986). It might be important for the Qi-site, as well as for the Qo-site, that YY260 and PEWY312 both lie in β -turns. Other conserved β -turns are at P40, at G91 just before the heme carrying span II, at K344 before span VII, and after span VII.

Cyt *b* from *Trypanosoma* is very different, showing not more than 25% identity with any of the other sequences (Table I). It is very rich in cysteins (Benne *et al.*, 1983). Special residues that are different from conserved positions in all the others, including the b_6f complexes, are: S40, F229, T300, S304, and I362, all instead of prolines; instead of aromats there are T118, C154, and LH198 instead of FF in helix V; a conserved tetrapeptide AFXS80 is SNWY in *Trypanosoma*. An important finding is that F105 in all the other bc_1 complexes is L in *Trypanosoma*. This F was thought to facilitate electron transfer between the two heme groups (Widger *et al.*, 1984), and was considered to explain the differences in the two types of complexes since it is replaced by M in cyt b_6 .

The value of comparing more and more sequences for exclusion of putatively important conservations is shown also by other examples: Plant mitochondria (Mz, Wh, and Oe) have a histidine at position 317 where all the others have an aromat. Another H is found at position 110 in the heme binding helix II, where the rest of the bc_1 complexes have an aromat, and the $b_6 f$ complexes have an I. Even more surprising is H at position 70 in wheat (Wh), where all the other sequences have an aromat. Could this be a mistake in sequencing? The same question arises for L92 in the sequence from toad (T).

Cyt b_6 + subunit IV is shorter at the C-terminus than cyt b. The hydrophobic span IX is missing. The bc_1 complexes show a conserved F427 in this span, which also might be involved in the difference at the Qi site between the two types of complexes. If span IV indeed is outside the membrane (see above), the cyt b-complement of the b_6f complex again might have the seven-helix motif generally postulated for proteins with transmembrane function (Dixon *et al.*, 1986). The N-terminus of subunit IV is highly charged, like the N-termini of cyt b. It contributes to a comparatively extended region between span V and VI in cyt b_6 + subunit IV which might additionally affect the Qi site. Interestingly, this extension is partially shared by bacterial cyt b,

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	M	79	88	100															
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bc ₁	¥	51	52	53	53	50	21	100											
•	Nc	55	56	57	57	53	22	61	100										
	A	54	56	58	57	55	24	61	86	100									
	Mz	53	56	55	55	54	24	54	55	56	100								
	Wh	54	56	55	55	54	24	54	54	55	99	100							
	0e	53	56	55	55	54	25	55	56	56	97	96	100						
	Pc	46	48	47	47	47	20	46	49	49	53	52	53	100					
	Rc	46	48	48	48	48	21	44	50	50	52	51	53	84	100				
	No	28	29	29	28	28	16	23	29	29	29	28	30	28	33	100			
h f	Lw	28	28	28	28	27	17	26	31	31	31	31	32	28	28	86	100		
~6*	ть	28	28	29	28	27	18	25	31	31	31	30	32	28	28	86	95	100	
	Sp	28	28	28	27	27	17	25	31	31	31	30	32	28	28	86	95	98	100

Table I. Percent Identity in Pairs of Cyt b/b_6 + IV (see Text for Description)

which additionally has an insertion after ILR321, close to the suggested Qo site between VI and VII. As pointed out before (Gabellini and Sebald, 1986), these insertions might reflect the lack of further subunits in plastidal and bacterial complexes.

Further, possibly important, differences between cyt b and $b_6 + IV$ are: b_6 has a positive charge, R99, next to the heme binding H98 in span II; there is IG instead of MS158 in III, K instead of T163, L182 instead of an aromat in IV, which interestingly also is L in *Schizosaccharomyces pombe* (Lang *et al.*, 1985; not shown), and R instead of H221. A difference emphasized before (Widger *et al.*, 1984; Nitschke and Hauska, 1987a) is the insertion of T202 after H201 in helix V. This additional residue between the two hemes must exert an additional helical torsion and might explain why cyt b_6 has a lower redox potential and is less stable than cyt b (Nitschke and Hauska, 1987a, 1987b).

Table I gives the percentage identities for the cyt b sequences counted from position 38 to 411 in Fig. 1, deletions included. The percentages are

similar to the ones presented before for a smaller number of sequences (Lang et al., 1985). The partial sequence (about 80%) for cyt b_6 + subunit IV from the cyanobacterium Nostoc (Malkin, personal communication; not shown in Fig. 1) has also been evaluated (No in Table I). The abbreviations are as for Fig. 1. The main observations are: (1) Cyt b_6 + subunit IV, including the cyanobacterial sequence, show about 30% identical residues to any other cyt b. (2) The protozoon Trypanosoma shows even less identity, which points to a very high mutation rate of mitochondrial genes in this parasitic organism with its special organization of the mitochondrial genome (Benne et al., 1983). Interestingly, cyt b from the related organism Leishmania tarantolae (de la Cruz et al., 1984) is 84% identical to the one from Trypanosoma. (3) Percentages of identity are significantly lower in vertebrate mitochondria than in plant chloroplasts, although species radiation occurred later, especially if one considers liverwort (Lw). (4) Even more pronounced is the higher conservation between cyanobacteria and plastids (86%) compared to bacteria and mitochondria (around 50%). Is the evolutionary clock of plastidal genes that come from cyanobacteria slower than the one of mitochondrial genes originating from bacteria? This is not the case because the sequences of mitochondrial cyt b of higher plants are as conserved as plastidal cyt b_6 (Table I). Probably plant organellar DNA has a comparatively slow mutation rate, possibly being better protected from mutagens like UV light. (5) Yeast is rather distant from filamentous fungi (Nc and A), as noted before (Lang et al., 1985).

How has cyt b evolved? Is there any indication of domain shuffling (Gilbert et al., 1986)? The splitting into cyt b_6 and subunit IV might indicate the evolutionary fusion of a heme-binding domain with a quinonereaction domain. Indeed, the cyt b gene of Schizosaccharomyces pombe shows an intron in this region (Lang et al., 1985), after K266. The gene in Saccharomyces cerevisiae is split by five introns, however. These are after Q161, G166, G186, G290, and, just before the conserved tetrapeptide, PEWY321 (Colson, 1987). The Neurospora gene is split twice, after G149 and E181, and the one from Aspergillus has an intron after G186, at the same site as the third intron of yeast (Burke *et al.*, 1984; Waring *et al.*, 1981). These introns separate the two heme binding helices II and V, reminiscent of the two peptides forming plastidal cyt b559 (Brown et al., 1982). So the origin from ancestral domains might be more complicated than indicated by cyt b_6 and subunit IV. Interestingly the N-terminal introns found in the plastidal genes of cyt b_6 and subunit IV (Shinozaki et al., 1986; Ohyama et al., 1986) are absent in the cyanobacterium (Kallas et al., 1986). There are no particularly conserved regions in cyt b, like in the Rieske FeS protein, although less identity is observed at the N- and C-terminus, as in other proteins.

The Rieske FeS Protein

The primary structure of the Rieske FeS protein from the $b_6 f$ complex of spinach chloroplasts has been obtained by sequencing genomic DNA (Steppuhn *et al.*, 1987). It confirms the partial sequence obtained directly from the protein (Pfefferkorn and Meyer, 1986). Figure 2 shows its alignment with three Rieske FeS proteins from mitochondria—beef = B (Shägger *et al.*, 1987), *Neurospora crassa* = Nc (Harnisch *et al.*, 1985), and yeast = Y (Beckmann *et al.*, 1987), and four from bacteria—*Paracoccus denitrificans* (Waring *et al.*, 1981), two strains of *Rhodobacter capsulatus* = Rc1 and Rc2 (Gabellini and Sebald, 1986; Davidson and Daldal, 1987a), and a partial sequence of *Rhodobacter sphaeroides* (Davidson and Daldal, 1987b). The arrangement and the indications of special residues and structures are as

в:	M?	75								
Nc:	MAPVSIVSRAAMRAAAAPARAVRALITTSTALQ									
Y:	MIGIRSSVRTCFKPMSLTSKRLISOSLLAS	KSTV								
Sp:	MIISIFNOLHLTENSSIMASFTISSATPSOLCSSKNCMFAPSIALAKAGRVNVLLSKERTRCMKLTCC-									
-										
		150								
		.								
B:	SHTDIKVPDFSDYRRPEVIDSTKSSKESSFARK	GFSVI VTATTTVCVAVAAKNUVSOFUMMEACA								
Nc:	ESPFKGESKAAKVPDFGKY-MSKAPPSTNMI	LESVENVGINGATTAAGAKSTTOEET KNMSASANVI								
Y:	RTPNFDOVLK-ENNDADKCR	SVAVENUCAMETI SSACAKSTVETETSCHTATAT								
Pc:	MSHADEHAGDHGATERI	DELYATACACTVAACAAAUTU VACANDCADYC								
Rc1:	MSHAEDNAGTRRI	DELYHATAATYSVVVTCAAVVVDT TAVARACATS								
Rc2:										
Rs:	WSNAEDHACTOP	DEL VYATACACAVATICAA								
Sn.		DI III TATAGAGAVATGAA VIPLINGANPSADVQ								
Dp.		AISIPADNYPHIOKRETIN								
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PC:	ALASIQVDVSGVETGIQUTVKVIGKPVETRRRTEDETQAL	SKEVDLGQLIDKSAQNSNKPDAPATDENRTMDEA								
RC1:	AMSSIF VDVSAVEVGTUITVKWRGKPVF1RRRDEKDIGL	MESVPIGALRUISAENANKPGAEATDENRSLAAFDG								
RCZ:	AMASIFVDVSAVEVGTULTVKWRGKPVFTRRRDKKDTEL	AKSYPIGALRUISAENANKPGAEATDENRTIPAFDG								
RS:	ALASIFVDVSSVEPGVQLIVKFIGKPIFIRRRTEADIEL	aksvoligolvD								
sp.:	LILIGALSLPIGYMLLPYASFIVPPGGGAGICGTLAKDAI	LGNDV LAAEWLKTHAPGDRTLTQGLKGDPTYLVVES								
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B:	KWVILIGVCIHLGCVPIAN-AGDFGGYYCPCHGSHY	TDASCRIRKGPAPINIEVPSYEFTSDGMVIVG								
NC:	EWLVMLGVCTHLGCVPIGE-AGDYGGWFCPCHGSHY	DISCRIBKGPAPINIEIPLYEFPEEGKLVIG								
¥:	OWLINIGICTHIGCVPIGE-AGDFGGWFCPCHGSHY	DISCRIRKGPAPINIETPAYEFDGD-KVIVG								
PC:	GEWLVMIGVCTHLGCVPIGDGAGDFGGWFCPCHGSHY	DISGRIRRGPAPONIHIPVAEFIDDTTIKLG								
Rc1:	TNTGEWLVMLGVCTHLGCVPMGDKSGDFGGWFCPCHGSHY	OSAGRIRKGPAPRNIDIPVAAFVDETTIKIG								
RC2:	TNTGEWLVHLGVCTHLGCVPMGDLSGDFGGWFCPCHGSHY	DSAGRIRKGPAPRNIDIPVAAFVDEITIKLG								
Sp.:	DKTLATIGINAVCIHLGCVVPFN-AAE-NKFICPCHGSQY	INNOGRVVRGPAPISIAIAHCD-VDDGKVVFVPW								
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Sp.: TEIDFRIGEAPWWSA 315

Fig. 2. Alignment of Rieske FeS protein (see text for description).

for Fig. 1. Identical residues (large dots) and conservative exchanges (small dots) are shown at the bottom for all eight sequences, on top for the seven bc_1 types.

The chloroplast protein (Sp) is considerably shorter at the N-terminus and longer at the C-terminus, but also the bacterial proteins are shorter at the N-terminus than the mitochondrial ones, which are rather variable in this region. In addition they have deletions after D200, in the middle part. The eukaryotic proteins are synthesized on cytoplasmic ribosomes, and leader peptides are shown in the first block of Fig. 2. The bovine leader peptide is not known because the sequence has been obtained directly from the mature protein (Schägger *et al.*, 1987). The double processing of the protein from *Neurospora* (Nc) on its path to its mitochondrial subcompartment (Hartl *et al.*, 1986), and of the chloroplast protein to the intrathylakoid space (Steppuhn *et al.*, 1987), will not be discussed here. Noteworthy is the fact that the leader peptide for the plastidal target is more than double the length of the mitochondrial counterparts. Perhaps this reflects the sorting of related protein precursors for different organelles in the plant cell. The bacterial Rieske FeS proteins probably lack leader peptides.

There are two hydrophobic regions, close to the N-terminus of the bc_1 types, each of them long enough to span the membrane. These might form a transmembrane hairpin (Schägger et al., 1987; see also Link et al. in Colson, 1987). The hydrophobicity is not very high, though, in line with the relatively easy dissociation of the Rieske FeS proteins from the complexes. Thus these proteins might actually not be transmembranous (see Gabellini and Neupert in Colson, 1987). Because of N-terminal shortening, the chloroplast protein shares only the second hydrophobic span. This is followed by a P and G-rich span instead of a highly positively charged stretch. Another hydrophobic region, found in all the cases, includes the conserved heptapeptide CTHLGCV249, which binds the Fe2S2 cluster from one side. The other side is bound to the conserved hexapeptide CPCHGS268 downstream, which probably forms a β -turn in a rather hydrophilic region, containing at least one additional β -turn. This highly conserved part has been pointed out before (Gabellini and Sebald, 1986) and additionally includes two aromats at positions 261 and 270, and one negative plus two positive charges at positions 257, 275, and 278. Further conserved features of possible significance are M143, the aromatic residue 172, after the N-terminal hydrophobic region, and a β -turn after D200, in the region of the mitochondrial deletions. The bc_1 types have additional aromats at positions 116, 118, 180, 258, 262, and 287. These might contribute to differences at the quinol oxidizing site Qo, resulting in different inhibitor sensitivities of the bc_1 and $b_6 f$ complexes (Hauska et al., 1983).

	bc ₁					b ₆ f
	В	NC	Y	Pc	Rc	Sp
В	100					
Nc	64 75	100100				
Y	63 ⁵³ 78	74 ⁶⁸ 83	100 100 100			
Pc	47 ³⁴ 68	49 ³⁶ 70	51 ³⁸ 72	100100		
Rc	44 ³³ 62	48 ³⁷ 68	48 ³⁷ ₆₇	69 ⁶² 82	100 100	
Sp	20 ₄₆	19 ₄₂	18 ₄₁	19 ₄₅	18 ⁴ ₄₂	100 100

 Table II.
 Percent Identity in Pairs of Rieske FES Proteins (see Text for Description)

The four cysteines holding the Fe2S2 center are shown by full arrows in Fig. 2. ENDOR measurements indicate that nitrogen is also a strong ligand to Fe in the bc_1 complex (Telser *et al.*, 1987). This could not be confirmed for the b_6f complex so far (Nitschke and Hauska, 1987a), but the two conserved histidines, one in each of the cluster binding peptides (open arrows), are very intriguing. These are absent in the Fe-binding peptides of ferredoxins which also otherwise are very different (Dayhoff, 1978; Smeekens *et al.*, 1985).

There is a clearly different degree of conservation in the N- and C-terminal part of the Rieske FeS proteins. This is documented in Table II, which gives the overall percent amino acid identities in large numbers, the one of the N-terminal part, from position 131 to 236, in superscripts, and the C-terminal one, from 237 to 296, in subscripts. Most striking is the difference when comparing the $b_6 f$ with the bc_1 types, but also, within the bc_1 types, the C-terminal part is less conserved. Possibly the Rieske FeS protein has different ancestral domains (Gilbert *et al.*, 1986), although no introns have been found so far.

Cytochrome c_1 or f

Figure 3 shows an attempt to align six, cyt c_1 -sequences, three mitochondrial—beef = B (Wakabayashi *et al.*, 1980), yeast = Y (Sadler *et al.*, 1984), and Neurospora crassa = Nc (Römisch et al., 1987)—and three bacterial ones—*Paracoccus* = Pc (Kurowski and Ludwig, 1987) and two strains of *Rhodobacter capsulatus* = Rc_1 , Rc_2 (Gabellini and Sebald, 1986; Davidson and Daldal, 1987a), with six cyt f-sequences—the partial sequence until 391 from the *cvanobacterium Spirulina* maxima = Sm (Alam and Krogmann. 1984), and five sequences from chloroplasts-liverwort = Lw (Ohyama et al., 1986), spinach = Sp (Alt and Herrmann, 1984), tobacco = Tb (Shinozaki *et al.*, 1986), pea (Willey *et al.*, 1984a), and wheat = Wh (Willey et al., 1984b). The sequences of beef and Spirulina have been obtained directly from the mature proteins, the rest from DNA. Organization of the figure and symbols are as for Fig. 1. The indication of complete identity (large dots), and of conservative exchanges (small dots), is shown on top for the bc_1 types, on the bottom for the $b_6 f$ types, and for all the sequences between Rc2 and Sm. Aromatic residues (asterisks) and charges are indicated on top for c_1 and on the bottom for f. Cyt c_1 from four species (Römisch et al., 1987), and of beef with cyt f (Davis, 1987), have been aligned before in a different way.

The first block shows the leader peptides. In Paracoccus this is followed by an enigmatic sequence of about 180 amino acids, highly acidic and rich in A, P, and T (Kurowski and Ludwig, 1987). The conserved heme-binding hexapeptide VCXXCH, which forms a β -turn close to the C-terminus, is indicated by arrows. Another feature found in all cases is the hydrophobic span at the C-terminus, probably forming a transmembrane helix stabilized by positive charges on either surface (Alt and Herrmann, 1984; Willey et al., 1984a). In addition, several β -turns occur in the same regions in cyt c_1 and f. Besides the heme-binding site only the region before the transmembrane helix shows some overall conservation, including the aromat F506. Further conservations comprise several aromats and charged amino acids, but overall identity is very low between cyt c_1 and f. Only 13 conservations and 21 conservative exchanges out of 308 residues are seen. As found also for the comparison of cyt b with cyt b_6 (see above), the plastidal protein Cyt f is much more conserved than cyt c_1 , although relatively less than cyt b_6 . This is compiled in Table III, which gives the percent pair identities from the N- to the C-terminus of Cyt f (position 248) to 556).

Cyt f shows several insertions, which in part are also found for bacterial cyt c_1 . In particular, the region around position 430, rich in β -turns, is partially identical between bacteria and chloroplasts.

Especially interesting is the lack of a conserved methionine in cyt f as a sixth ligand to the heme-Fe. This M is at position 477 for cyt c_1 (arrow). The only conserved M in cyt f is at the C-terminus, spaced from the heme by the membrane. An absorption of oxidized cyt f around 690 nm, characteristic for

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Pc:	HTLRNASLITAVAALITVALAGGAVAQDASTAPGTTAPAGSSY	************************	HINEA
Rcl:	MKKLLISAVSALVIGSGAALA		
Rc2:	MKKLLISAVSALVLGSGAAFA	**********************	
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NC:		DAXBEACTER SEALSEAD	ALVGTILTV
Pc:	DHGDAAAQEAGDSHAAAHIEDISFS-FEGPFGKFDQHQLQRGL	WYTEVCSACHGLRYVPL	TLADEGGPO
Rc1:	NSNVODHAFS-FE-GIFGEFDO-AOIREGFO	VYSEVCSTCHGMKFVP1	TISDDGGPO
Rc2:	GIEGKYDO-AOLBRGED	VVNEVCSACHCHKEVPTE	TADDCCPO
Cm ·	VDEWA	TYPETYCANCET AAKDTET	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
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TD:		-ICRIVCANCHLANKPVE1	EVPQ-AVLP
Pea:	QQ-GYENPREA-	-IGRIVCANCHIANKPVD1	EVEQ-AVLP
Wh:	QQ-GYENPREA-	-TGRIVCANCHLASKPVD1	EVPO-AVLP
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¥:	EE-VRNMAEEFEYDDEPDEQGNPKKRPGKISDYIPGPYP-NEQ	ARAANQGALPP-DISLIY	KARH
Nc:	DE-AKALAEENEYDTEPNDQGELEKRPGKLSDYLPDPYK-NDE	ARFANNGALPP-DLSLIV	KARH
Pc:	LPEDQVRA-YAANF-DIDTP-ETEEDRPRVPTDHFP	TVSGEGMGP-DLSLM2	KARAGFHGP
Rc1:	LDPTFVRE-YAAGIDTIIDKDSGEERDRKETTMFP	TRVGDCHGP-DLSVIP	KARAGESGP
Rc2	TDPTRVRR-VAACIIVIT TORDSGERRDRRETTERP		RAPACESCE
Smi	DEVERSIONET DUDE- NT OOVI COCS-ECAT NUCLUT DAT DOVER	TADADOT -DEFNAFEWAR	VENDVCA
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LW:	THE	TITITITI TITI	FUPTONIK
sp:	UTVFKAVVRTPYURQLKQVLANGK-KGGLNVGAVL-ILPEGFEI	APPDRISPENK-EKNON	SFOSYRPAK
Tb:	DIVERAVVRIPYDEQIKQVLANGK-RGGLNVGAVL-ILPEGERI	APPDRISPEME-ERIGNI	SENSTREME
Pea:	DIVFEAVORIPTOHOVROVIANGK-KGAINVGAVIILPEGPEI	APPHRISPOIK-ERIGNI	SFOSYRPTK
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Fig. 3. Alignment of cyt c_1/f (see text for description).

the sulfur ligation of heme c (Yu *et al.*, 1986), is seen, although with unusual shape (W. Nitschke, unpublished). The sixth heme ligand of cyt f thus is unclear.

Conclusions

1. Cyt b is the most highly conserved of the three redox center-carrying subunits of cyt bc_1/b_6f complexes. It probably is of monophyletic origin. Cyt b_6 + subunit IV of cyanobacteria and of chloroplasts resulted from gene splitting, or formed cyt b by gene fusion. They are very distant to any other cyt b, with less than 30% identity. This evolutionary distance is also

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в:	ĢĢ <u>k</u> D¥VPS	LITGYC-EPPI-GVS	REGLYFNPYF-		
Y:	GGCDYIPS	LLTGYPDEPPA-GVA	LPPGSNYNPYP-		
Nc:	GGCDY1PS	LLITGYPDEPPA-GAST	VGAGLNFNPYF-		
Pc:	YGTGLSQLFNGIGGPEYIHA	VLTGYDGEEKEEAGA	VL-YHNAAF-		
Rc1:	AGSCHNOLFKGIGGPEYTYR	YVIGFPEENPACAPE	GIDGYYY——NEVF∙	ΩVĢĢV₽DŢÇKDAAĢI	
Rc2:	AGSGANQLPKGMGGPEYIYN	IVIGE-EENPECAPE	GIDGYYYNKTF	-QIGGVPDICKDAAGV	
Sm:	ENVVIVGPIPGDQYSE				
Lw:	KYILVIGPVPGKKYSEMVFP	ILSPOPATNKEAHFL	KYPIYVGGNRGR	QIYPOGSKSNNTV	YNASITCKVS
Sp:	QUILVIGPVPGQKYSEITFP	LIAPOPATKKOVHFL	KYPIYVGGNRGR	QIYPOCSKSNNIV	YNSTATCIYK
Tb:	KNILVIGPVPGQKYSETTPP	II SPOPATKKOVHFL	KYPIYVGGNRGR	QIY PDGSKSNNIY	YNATAAGIVS
Pea:	KNILVIGPVPGKKYSEITTP	II.SPDPATKROVYFIJ	KYPLYVGGNRGR	QTYPDGSKSNNNV	SNATATOVYK
Wh:	KNILVIGPVPGKKYSEIVPP	ILSPOPATKKDAHFLI	KYPIYVGGNRGR	GQIYPDGSKSNNTV	YNATSTGIVR
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B:	PGQAI	GMAPPIYNEV	LEFDÖCTPATMSOV	AKÖVCTFLRWAAEPEH	DH-RKRMGLK
Y:	PGGS1	AMARVLFDDM	VEYEDCTPATTSOM	ARDVITTFLNWCAEPER	DE-RKRICLK
Nc:	PGTG1	AMARVLYDGL	VDYEDGTPASTSO	ROVVEFINWAAEPEN	DD-RKRMCMK
Pc:	AGNW1	OHAAPISDDO	VTYEDGTPATVDOM	ATOVAAFINITAEPKI	MD-BROVGFV
Rc1:	KITHGSWA	OHPPALFDOL	VIYEDGIPATVDO	GODVASFIMMAAEPKI	VA-BROMGLY
Rc2:	KITHGSWA	RIPPPLVDDQ	VIYEDGTPATVDOM	AQDYSAFLMMAAEPKI	VA-RRONGLV
Lw:	KIFRKERGGYEITIDDISDG	HKVVDISAAGPELII	SEGELVKVDOPLTN	NPNVGGFGOGDAEVVI	ODPIRIOGIL
SD:	KIVRKERGGYKINIADASDG	REVVDIIPRGPELLV	SECRETIKLDOPLIS	FINGEFGOGDAEVVI	ODPLRIOGUT.
TD:	KLIRKERGGYEITITDASDG	ROVVDIIPEGPELLV	SECES I REDOPLIS	PHYGGEGOGDAEIVI	ODPLRVOGLT.
Pea:	ÖLLRKENGGYELTIVDASDG	SEVIDIIPPGPELLY	SEGESTRIDOPTIS	PNVGGFGOGDAFIVI	ODPLRVOGTT.
Wh:	KILRKENGGYEISIVDASDG	ROVIDIIPPGPELLV	SEGESTRUDOPUTS	PNVGGFGOGDAFIVI	ODPLRVOGIT.
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B:	MLLMMGLLLPLVYAMKRHRW	SVLKSRKLAYRPPK-			
Y:	TVITISSINTISIWVKKFKW	AGIETREFVENPPEP	R K -		
NC:	VI VVISVI FALSVYVKRYKW	WIRSPRIVYDPPRR	PPPATNI AL POORAL	KS	
Pct	SVIFLIVI AALLYLINK-KI	OPTKHPRKRNDRPV	LIKRIRGAFFFAG	FPFAL PDMDKT	
Rc1:	AVVMIGT SVMLYLINK-RT	APYKROKA			
Rc2:	AMVMIGUS VMUNUMK-RU	APYKONKA			
Tw:	TFPGSVITAOTFINIKK-KO	FERVOLAEMNE			
Sn:	PETASVILAOUTIVIAS-80	FERVOLSEMNP			
Th:	PPLASVILAOUPLVIAK-KO	PERVOLATION			
Pea	IFTASTITAOTILIVIAK-KO	PERVOI SEMME			
Wh:	PPPASVILAOVELVI KK-KO	PERVOLVEMNE			
	* 69.6	****			

Fig. 3. Continued.

documented by cyanobacterial cyt c_6 and mitochondrial cyt c (Dayhoff, 1978). Noteworthy is the fact that the β -subunit of the coupling factor complex shows over 80% identity between chloroplasts, mitochondria, and bacteria (Walker *et al.*, 1985). This cannot be merely explained by higher functional stringency, because even then the evolutionary distance should be seen, but is not.

2. Cyt *b* of plant mitochondria, like cyt b_6 + subunit IV of chloroplasts, is significantly more conserved than cyt *b* of animal mitochondria, although cyt *c*, which is encoded in the nucleus, is similarly conserved within plants and animals (Dayhoff, 1976). Thus the organellar genomes of plants change more slowly than the nucleus, which is the opposite from animals (Brown *et al.*, 1982).

3. Cyt b_6 + subunit IV, as well as cyt f, are more closely related in cyanobacteria and chloroplasts than cyt b and cyt c_1 from bacteria and mitochondria.

4. Cyt c_1 and Cyt f arose by convergence of different ancestral proteins.

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	В	Y	NC	Pc	Rc	Sm	Lw	Sp	Тb	Pea	Wh
В	100										
Y	59	100									
Nc	61	66	100								
Pc	26	35	32	100							
Rc	26	30	28	53	100						
Sm	9	8	8	8	7	100					
Lw	8	10	9	11	8 7 1 (1 13 (63	100				
Sp	8	11	9	10	12	64	84	100			
Tb	9	12	9	11	13	65	83	94	100		
Pea	8	11	9	11	13	62	81	91	91	100	
Wh	8	11	9	10	12	64	85	95	93	90	100
	B Y NC PC RC Sm Lw Sp Tb Pea Wh	B B 100 Y 59 Nc 61 Pc 26 Rc 26 Sm 9 Lw 8 Sp 8 Tb 9 Pea 8 Wh 8	B Y B 100 Y 59 100 Y 59 100 Nc 61 66 Pc 26 35 Rc 26 30 Sm 9 8 Lw 8 10 Sp 8 11 Tb 9 12 Pea 8 11 Wh 8 11	B Y Nc B 100 100 Y 59 100 Nc 61 66 100 Pc 26 35 32 Rc 26 30 28 Sm 9 8 8 Lw 8 10 9 Sp 8 11 9 Tb 9 12 9 Pea 8 11 9 Wh 8 11 9	B Y Nc Pc B 100	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$

Table III. Percent Identity in Pairs of Cyt c_1/f

5. The Rieske FeS proteins have a common origin of the C-terminal part, only.

6. All three proteins carry insertions in chloroplasts and bacteria that might compensate for the lack of the additional sununits present in mitochondria.

7. The peptides carrying the redox centers can be defined for all three proteins. In addition, conserved aromatic residues indicate the sites for electron transfer, in particular with quinone/quinol. These putative reaction sites in cyt b, as well as the redox centers of cyt c_1 or f, and of the Rieske FeS protein, are formed by β -turns of the peptide backbone.

8. Differences in the conserved structures of bc_1 and b_6f -polypeptides are observed and might explain the different inhibitor sensitivity.

9. We believe that this comparative approach could finally lead to a realistic, detailed model of the $bc_1/b_6 f$ complexes, when extended by computeraided secondary structure predictions and combined with experimental test.

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References

- Alam, J., and Krogmann, D. W. (1984). In Advances in Photosynthesis Research, Vol. 1 (Sybesma, C., ed.), Martinus Nijhoff/Dr. W. Junk Publishers, Dordecht, Netherlands, pp. 1.4.521–524.
- Alt, J., and Hermann, R. G. (1984). Curr. Genet. 8, 551-557.
- Anderson, S., Bankeir, A. T., Barrell, B. C., de Bruijn, M. H. L., Coulson, A. R., Dronin, J., Eperon, I. C., Nierlich, D. P., Roe, B. A., Sanger, F., Schrier, P. H., Smith, A. J. H., Staden, R., and Young, I. G. (1981a). *Nature (London)* 290, 456–465.
- Anderson, S., de Bruijn, M. H. L., Coulson, A. R., Eperon, I. C., Sanger, F., and Young, I. G. (1981b). J. Mol. Biol. 156, 683–717.
- Beckmann, J. D., Ljungdahl, P. O., Lopez, J. L., and Trumpower, B. L. (1987). J. Biol. Chem. 262, 129–000.
- Benne, R., De Vries, B. F., Van den Burg, J., and Klaver, B. (1983). Nucleic Acids Res. 11, 6925-6941.
- Bibb, M. J., Van Elten, R. A., Wright, C. T., Walberg, M. W., and Clayton, D. A. (1981). Cell 26, 167-180.
- Boer, P. H., McIntosh, J. E., Gray, M. W., and Bonen, L. (1985). Nucleic Acids Res. 13, 2281–2292.
- Brandle, C. J., and Deber, C. M. (1986). Proc. Natl. Acad. Sci. U.S.A. 83, 917-921.
- Brown, W. M., Prager, E. M., Wang, A., and Wilson, A. C. (1982). J. Mol. Evol. 18, 225-239.
- Burke, J. M., Breitenberger, C., Heckman, J. E., Dujon, B., and Rajbhandary, U. L. (1984). J. Biol. Chem. 259, 504-511.
- Chou, P. Y., and Fasman, G. D. (1979). Biophys. J. 26, 367-383.
- Colson, S. (1987). In Cytochrome Systems: Molecular Biology and Bioenergetics (Papa, S., ed.), Proc. Int. Symp. 1987, Fasano, Plenum Press, New York, in press.
- Crofts, A. R. (1985). In *The Enzymes of Biological Membranes* (Martonosi, A. N., ed.), Plenum Press, New York, pp. 347–382.
- Davidson, E., and Daldal, F. (1987a). J. Mol. Biol. 195, 13-24.
- Davidson, E., and Daldal, F. (1987b). J. Mol. Biol. 195, 23-30.
- Davis, D. J. (1987). Progress in Photosynthesis Research (Biggins, J., ed.), Martinus Nijhoff Publishers, Dordrecht, Netherlands, pp. II.7.473-476.
- Dawson, A. J., Jones, V. P., and Leaver, C. J. (1984). EMBO J. 3, 2107-2113.
- Dayhoff, M. O. (1976). Atlas of Protein Sequence and Structure, Suppl. 2, Natl. Biomed. Res. Found., Washington, D.C., p. 33.
- Dayhoff, M. O. (1978). Atlas of Proetin Sequence and Structure, Suppl. 3, Natl. Biomed. Res. Found., Washington, D.C.
- de la Cruz, V. F., Neckelmann, N., and Simpson, L. (1984). J. Biol. Chem. 259, 15136-15147.
- Dixon, et al. (1986). Nature (London) 321, 75-79.
- Gabellini, N., and Sebald, W. (1986). Eur. J. Biochem. 154, 569-579.
- Gilbert, W., Marchionni, M., and McKnight, G. (1986). Cell 46, 151-154.
- Harnisch, U., Weiss, H., and Sebald, W. (1985). Eur. J. Biochem. 149, 95-99.
- Hartl, F. U., Schmidt, B., Wachter, E., Weiss, H., and Neupert, W. (1986). Cell 47, 939-951.
- Hauska, G. (1985). In Molecular Biology of the Photosynthectic Apparatus (Steinback, K. E., Bonitz, S., Arntzen, C. J., and Bogorad, L., eds.), Cold Spring Harbor Laboratory, pp. 79–87.
- Hauska, G. (1986a). Encycl. Plant Physiol., New Ser. 19, 496-507.
- Hauska, G. (1986b). Methods Enzymol. 126, 271-285.
- Hauska, G., Hurt, E., Gabellini, N., and Lockau, W. (1983). Blochim. Biophys. Acta 726, 97-133.
- Heinemeyer, W., Alt, J., and Herrmann, R. G. (1984). Curr. Genet. 8, 543-606.

- Helmer-Citterich, M., Morelli, G., and Macino, G. (1983). EMBO J. 2, 1235-1242.
- Herrmann, R. G., Alt, J., Schiller, B., Widger, W. R., and Cramer, W. A. (1984). FEBS Lett. 176, 239-244.
- Kallas, T., Spiller, S., and Malkin, R. (1986). Progress in Photosynthesis Research (Biggins, J., ed.), Martinus Nijhoff Publishers, Dordrecht, Netherlands, pp. IV.12.801-803.
- Koike, K., Kobayashi, M., Yaginuma, K., Taira, M., Yoshida, E., and Imai, M. (1982). Gene 20, 177-185.
- Kurowski, B. and Ludwig, B. (1987). J. Biol. Chem. 262, 13805-13811.
- Lang, B. F., Ahne, F., and Bonen, L. (1985). J. Mol. Biol. 184, 353-366.
- Ljungdahl, P. O., Pennoyer, J. D., and Trumpower, B. L. (1986). Methods Enzymol. 126, 181-190.
- Markos, A., Blahuskova, A., and Kalous, M. (1986). 4th Eur. Bioenerg. Conf. (EBEC), Prague, 1986, reports, Vol. 4, 35.
- Nitschke, W., and Hauska, G. (1987a). Progress in Photosynthesis Research (Biggins, J., ed.), Martinus Nijhoff Publishers, Dordrecht, Netherlands, Vol. II, pp. 165-172.
- Nitschke, W., and Hauska, G. (1987b). Biochim. Biophys. Acta, 892, 314-319.
- Nobrega, F. G., and Tzagoloff, A. (1980). J. Biol. Chem. 255, 9828-9837.
- Ohyama, et al. (1986). Nature (London) 322, 572-575.
- Pfefferkorn, B., and Meyer, H. E. (1986). FEBS Lett. 206, 233-237.
- Pfefferkorn, B., and Meyer, H. E. (1987). Hoppe-Seylers Biochem. 368, 561.
- Phillips, A. C., and Gray, J. C. (1984). Mol. Gen. Genet. 194, 477-484.
- Rich, P. (1984). Biochim. Biophys. Acta 768, 53-79.
- Robertson, D. E., Davidson, E., Prince, R. C., van den Berg, W. H., Marrs, B. L., and Dutton, D. L. (1986). J. Biol. Chem. 261, 584–591.
- Roe, B. A., Ma, D. P., Wilson, R. K., and Wong, J. F. H. (1985). J. Biol. Chem. 260, 9759-9774.
- Römisch, J., Tropschung, M., Sebald, W., and Weiss, H. (1987). Eur. J. Biochem., in press.
- Sadler, I., Suda, K., Schatz, G., Kaudewitz, F., and Haid, A. (1984). *EMBO J.* **3**, 2137–2143. Saraste, M. (1984). *FEBS Lett.* **166**, 367–372.
- Schägger, H., Link, T. A., Engel, W. D., and von Jagow, G. (1986). *Methods Enzymol.* 126, 224–237.
- Schägger, H., Borchart, U., Machleidt, W., Link, T. A., and von Jagow, G. (1987). FEBS Lett., in press.
- Schuster, W., and Brennicke, A. (1985). Curr. Genet. 9, 157-163.
- Shinozaki, et al. (1986). EMBO J. 5, 2043-2049.
- Smeekens, S., van Binsbergen, J., and Weisbeek, P. (1985). Nucleic Acids Res. 13, 3179-3194.
- Steppuhn, J., Hermans, J., Janson, T., Vater, J., Hauska, G., and Herrmann, R. G. (1987). Mol. Gen. Genet., in press.
- Telser, J., Hoffman, B. M., LoBrutto, R., Ohnishi, T., Tsai, A. L., Simpkin, D., and Palmer, G. (1987). FEBS Lett. 214, 117–121.
- Wakabayashi, S., Matsubara, H., Kim, C. H., Kawai, K., and King, T. E. (1980). Biochem. Biophys. Res. Commun. 97, 1548–1554.
- Walker, J. E., Fearnley, I. M., Gray, N. J., Gibson, B. W., Northrop, F. D., Powell, S. J., Runswick, M. J., Saraste, M., and Tybulowicz, V. L. Y. (1985). J. Mol. Biol. 184, 677-701.
- Waring, R. B., Davis, R. W., Lee, S., Grisi, E., McPhail-Berks, M., and Scazzocchio, C. (1981). Cell 27, 4–11.
- Widger, W. R., Cramer, W. A., Herrmann, R. G., and Trebst, A. (1984). Proc. Natl. Acad. Sci. USA 81, 674-678.
- Willey, D. L., Auffret, A. D., and Gray, J. C. (1984a). Cell 36, 555-562.
- Willey, D. L., Howe, C. J., Auffret, A. D., Bowman, C. M., Dyer, T. A., and Gray, J. C. (1984b). *Mol. Gen. Genet.* 194, 416–422.
- Yu, L., Dong, J. H., and Yu, C. A. (1986). Biochim. Biophys. Acta 852, 203.