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The Chloroplast Envelope: Is It Homologous With the Double Membranes of Mitochondria and Gram-Negative Bacteria?

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Chloroplasts of higher plants possess three separate membranes. In addition to the internal thylakoid membranes, which are involved in the light reactions of photosynthesis, chloroplasts are surrounded by a pair of lipid bilayer membranes called the envelope. Although the envelope membranes have not been studied as intensively as the thylakoid membranes, there is evidence that envelope membranes have several important functions. They mediate metabolite transport between the stromal space of the chloroplast and the cytoplasm of the cell [1]. They are also important in chloroplast biogenesis. For example, the envelope membranes play a major role in the biosynthesis of galactolipids, the predominant lipid in all chloroplast membranes [2]. In addition, the envelope mediates the import of cytoplasmically synthesized proteins into chloroplasts [3].

Chloroplasts are not unique in being surrounded by a pair of lipid bilayer membranes. Mitochondria and Gram-negative bacteria are also bounded by two membranes. However, the mitochondrial and bacterial membranes are much better characterized than the chloroplast envelope membranes. Thus, these two can serve as valuable model systems for the chloroplast envelope. Our major purposes in this review are twofold: first, to make comparisons among the bounding double-membrane systems of mitochondria, Gram-negative bacteria, and chloroplasts; second, to review recent advances in our understanding of the chloroplast envelope membranes. No effort is made to provide comprehensive coverage of any of the membrane systems; rather, the focus is on homologies among the various systems that provide important insights into the structural and functional properties of the chloroplast envelope.

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OVERALL STRUCTURAL FEATURES

The diagram shown in Figure 1 depicts the general structural features of chloroplasts, mitochondria, and Gram-negative bacteria. As noted above, each is surrounded by a pair of lipid bilayer membranes. However, the double membranes in each system have certain unique features. In Gram-negative bacteria the two membranes are separated by a layer of peptidoglycan, which provides cell shape and rigidity [4]. In addition, the bacterial outer membrane contains a high proportion of lipopolysaccharide as well as the usual phospholipid and protein components [4]. In mitochondria, the inner membrane has extensive infoldings, which greatly increase the surface area of this membrane. This infolding may also lead to lateral heterogeneity of the inner membrane. It has been suggested that the infolded regions, called "cristal membrane," are structurally and functionally distinct from the "inner boundary membrane" that lies parallel to the outer membrane [5,6]. In chloroplasts the envelope appears as a pair of membranes, separated by 5–10 nm, and running parallel around the entire perimeter of the chloroplast. Chloroplasts also have an internal membrane system, the thylakoids. The thylakoid membranes may be related ontogenetically to the inner envelope membrane as there is evidence to suggest that vesicles derived from the inner envelope contribute to the biogenesis of thylakoids [7,8]. However, the inner envelope membranes and thylakoids are each clearly distinct in composition and function [2].

Although important structural differences exist among the three systems, certain similarities are apparent. For example, in each of the systems, the two membranes differ dramatically in their permeability properties. Invariably, the outer membrane is nonspecifically permeable to small molecules and the inner membrane is the permeability barrier and the site of specific translocators of various ions and metabolites [9–14].

The permeability of the outer membrane of chloroplasts was demonstrated several years ago by Heldt and Sauer [9]. They placed chloroplasts in solutions containing different concentrations of sorbitol and observed the effects by electron microscopy. Under hypertonic conditions, the inner envelope membrane plasmolyzed away from the outer, leaving extra space between the two membranes. They used radioactive compounds to confirm that several different small molecules had free access to this intermembrane space.

At present, relatively little is known concerning the mechanism by which the chloroplast outer envelope membrane is permeable to small molecules. However, in mitochondria and Gram-negative bacteria, specific outer membrane polypeptides, porins, form pores through the outer membrane [10–12]. Mitochondria appear to contain a single type of porin, whereas many bacterial species contain more than one porin type [10,12,14]. Considerable characterization of the mitochondrial and bacterial porins has been conducted. For example, in each system they have been identified, purified, and reconstituted into lipid bilayer membranes to reform pores similar to those of the original membrane [10,12–14]. In general, the porins of enteric bacteria have a molecular weight limit for permeant molecules of 600–800 daltons [10], whereas the porins of mitochondria and other species of bacteria allow molecules up to several thousand daltons to pass [10,13,14]. There is also evidence that the mitochondrial porin forms a voltage-dependent pore, suggesting a possible mechanism for regulating the permeability of the outer membrane [12]. By analogy with these systems, it seems likely that the chloroplast outer membrane also contains a

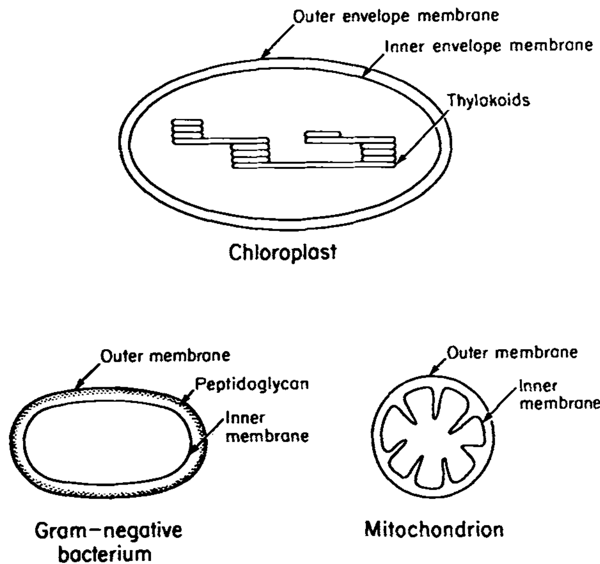

Fig. 1. Diagrammatic representation of the membranes of chloroplasts, Gram-negative bacteria, and mitochondria.

pore-forming polypeptide. The identification, purification, and characterization of this polypeptide provide important areas for further research on the chloroplast outer envelope membrane.

A second area of structural similarity among the three systems is the existence of adhesion zones, or contact sites, between the two membranes. These contact sites are regions where the two membranes appear to be held in close proximity by some special interactions [15,16]. Although these structures are poorly understood in all three systems, there is growing evidence that they are physiologically important. The postulated roles of contact sites in mediating interactions between the two membranes are discussed in more detail later in the review. Contact sites may present special problems during efforts to separate and purify the two membranes. These problems are discussed in the next section.

ISOLATION OF CHLOROPLAST ENVELOPE MEMBRANES

Biological membranes are intricate structures with complex metabolic functions. Investigation of the structure and function of any biological membrane requires the ability to isolate it in a highly purified state. For the membranes of bacteria, mitochondria and chloroplast envelopes, purification of each membrane free of the other is not a simple task. The physical proximity of the two membranes in situ can lead to fusion during lysis and to the formation of vesicles of one membrane trapped within vesicles of the other. This problem is compounded by the presence of contact sites or adhesion zones, which appear to mediate special interactions between the two membranes.

In the mid to late 1960s, several different procedures were developed which allowed the separation of the two mitochondrial membranes [17,18]. About the same time, Miura and Mizushima reported the first separation of the two membranes of Escherichia coli [19]. In each of these cases the initial reports of separation procedures were followed by further improvements and refinements in the procedures [17,20,21]. More importantly, the ability to isolate each membrane allowed detailed characterization of the structure and function of each membrane as well as investigations into the interactions between the two membranes [4,17,22].

The development of procedures for separating the two membranes of the chloroplast envelope have lagged behind those for mitochondria and bacteria. A procedure for isolating the chloroplast envelope relatively free of internal thylakoids was first reported by Mackender and Leech in 1970 [23]. Douce et al subsequently reported a modified procedure that resulted in envelope preparations completely free of thylakoid contamination [24]. These procedures, as well as those reported by others [25], yield envelope preparations that contain a mixture of the two envelope membranes [25,26]. Unfortunately, it has not been possible to further fractionate such preparations into inner and outer envelope membranes [26; Keegstra et al, unpublished observations]. The inability to subfractionate the membranes may result from the method used to rupture the chloroplasts during envelope isolation. The initial envelope isolation procedures rupture chloroplasts by suspending them in hypotonic buffer containing Mg^{+2}, causing the chloroplasts to swell and burst. Thus, the envelope membranes are pressed tightly against each other at the time of lysis. The result is that a large amount of membrane material ends up as vesicles within vesicles (double-membrane vesicles), presumably one vesicle of inner and one of outer envelope membrane [25,27]. In addition, there may be fusion of inner and outer membrane fragments to yield mixed vesicles, as has been suggested by Joyard and Douce [26]. Regardless of the mechanisms involved, the final result is a mixture of envelope membranes which are not separable.

Although much valuable information about the chloroplast envelope has been gained by investigating the mixture of two membranes [2], the inability to subfractionate them has been a limitation to further studies on the properties and functions of each membrane. This prompted us to investigate alternate procedures for isolating chloroplast envelopes which would permit subsequent separation of the two membranes. Recently we reported such a method [27]. A flow diagram of the steps in the isolation procedure is shown in Figure 2. The most important differences from previous methods are the conditions of lysis. As shown in Figure 2, suspension of chloroplasts in hypertonic buffer results in physical separation of the two envelope membranes. In pea chloroplasts the absence of Mg^{+2} is necessary to optimize this physical separation and to minimize subsequent cross contamination of the two membranes. The reason for this effect is uncertain, but Mg^{+2} may form salt bridges between negative charges on the two membranes. If chloroplasts are ruptured under these hypertonic conditions in the absence of Mg^{+2}, the regions of separated membranes give rise to single membrane vesicles which subsequently can be purified to yield inner and outer membrane. However, even under hypertonic conditions there are regions where the two membranes appear not to separate [9,28]. These regions, containing the putative contact sites, presumably give rise to the double membrane vesicles which are present in certain purified envelope fractions (see discussions below).

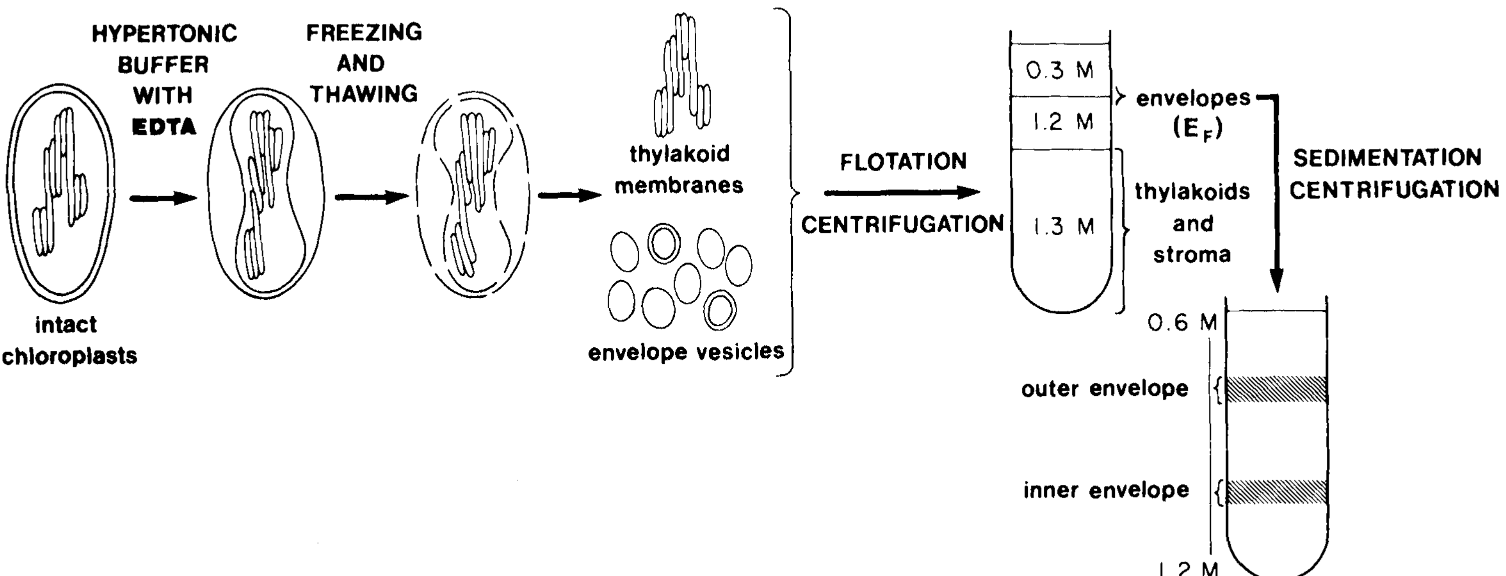

Fig. 2. Flow diagram of a method for purifying the outer and inner membranes of the chloroplast envelope. Details of the procedure are given in Cline et al [27].

If chloroplast rupture is carried out under the proper conditions the other steps in the procedure can be varied without adverse effects. For example, several methods of chloroplast rupture can replace the cycle of freezing and thawing shown in Figure 2. These include the use of a glass homogenizer or passage of the chloroplasts through a small pore nylon mesh [Keegstra et al, unpublished results].

The procedure has been used primarily with pea chloroplasts, but it is expected that it will have general application to other chloroplasts. In fact, Dorne et al [29] and Block et al [30] have used a modification of the procedure shown in Figure 2 to separate the inner and outer envelope membranes from spinach chloroplasts. Unfortunately the details of their modifications have not been published at the time of this writing, so it is not possible to determine whether their modifications represent an improvement over our present procedures [27].

Although the procedure shown in Figure 2 resolves the two envelope membranes, cross contamination is still a problem [27,31]. The outer envelope membrane fraction is virtually free of inner membrane, but the inner membrane fraction has considerable contamination by outer membrane (Table I). The inner membrane region of the second gradient (Fig. 2) is heterogeneous with respect to the extent of contamination by outer membrane. The upper portion of the band contains significant amounts of outer membrane, including substantial quantities of double membrane vesicles which retain the close spacing of membranes seen in intact chloroplasts [27,28,31]. The lower portion of the inner membrane band is the purest, but still contains some outer membrane [27,28,31]. Similar cross contamination problems have been observed repeatedly in efforts to purify the two membranes of mitochondria and Gram-negative bacteria (Table I). With mitochondria, mutual cross contamination of 5-20% is observed depending upon the method of isolation employed and the source of the mitochondria [20,32].

The cross contamination is an interesting problem, because in all three systems the two membranes have very different densities and should separate cleanly (Table II). It has been suggested that the cross contamination with mitochondria is due to the

TABLE I. Cross Contamination in Double-Membrane Systems*

		Contamination by[a]—	
		Outer Membrane (%)	Inner Membrane (%)
Chloroplast envelope (peas)	Outer membrane	—	<1.5
	Inner membrane	9	—
Mitochondria (yeast)	Outer membrane	—	18.4
	Inner membrane	10.6	—
Bacteria (*Salmonella typhimurium*)	Outer membrane	—	<3
	Inner membrane	10	—

*Contamination of chloroplast envelope taken from [31,75] of mitochondrial subfractions from [32], and of bacterial subfractions from [21].
[a]Values reported on a protein basis.

TABLE II. Properties and Functions of Double-Membrane Systems

	Chloroplast envelope[a]		Mitochondria[b]		Gram-negative bacteria[c]	
	Outer membrane	Inner membrane	Outer membrane	Inner membrane	Outer membrane	Inner membrane
Properties						
Buoyant density	1.08	1.13	1.07–1.13	1.14–1.21	1.22	1.15
Lipid composition	Galactolipid/ phospholipid ~1.1	Galactolipid/ phospholipid ~4:1	Phospholipids	Phospholipids	Lipopolysaccharide/ phospholipids	Phospholipids
Functions						
Lipid synthesis	+	+	+	+	No	+
Ion and metabolite transport	Permeable to small molecules	Specific translocators	Permeable to small molecules (protein pore)	Specific translocators	Permeable to small molecules (protein pore)	Specific translocators
Respiratory electron transport and ox-phos	No	No	No	+	No	+
Translocation of lipids and proteins	+	+	+	+	+	+

[a]Information on buoyant density and lipid composition were obtained from [27], lipid synthesis and transport from [2,31,44,75], and the remaining information from [2].
[b]Buoyant density values were from [22], lipid compositions and synthesis from [22,43], and the remaining information from [17,22].
[c]Buoyant density values and lipid compositions were obtained from [21], lipid synthesis from [41,42], metabolite transport from [76], and lipid and protein translocation from [15].

presence of stable contact sites that mediate interactions between the two membranes in vivo and then survive to hold the two membranes together during isolation procedures [16]. This may also be the case with bacteria. Bayer et al have reported some success in identifying membrane fractions that are enriched in putative adhesion zones [33]. As expected these fractions have characteristics of both outer and inner membranes and are intermediate in density between the two purified membranes.

We have recently examined this problem in chloroplasts using freeze-fracture electron microscopy [28]. In these experiments isolated chloroplasts were incubated in hypertonic 0.6 M sucrose-EDTA to expose areas of contact. Under these conditions the contact sites become aligned and appear as grooves in the E-face of the outer membrane. Interestingly, these grooves are lined with intramembranous particles, probably proteins. We suspect that these particles mediate the attachment between the two envelope membranes. Similar particle-lined grooves can be seen in isolated double-membrane vesicles, which contain both inner and outer membrane. Additional work needs to be done to extend these observations and to identify the putative polypeptides involved in forming contact sites. Nevertheless, this work provides evidence to support the hypothesis that contact sites which may mediate interactions between the two envelope membranes in vivo may also contribute to the cross-contamination problem. It should be noted that if this interpretation is correct and contact sites are causing portions of the outer membrane to associate with inner membrane, the material isolated as outer membrane may be only a subfraction of the complete outer membrane. The contact sites and other outer membrane components associated with them may not be present in isolated outer membrane vesicles, but only in the outer membrane fragments associated with inner membrane.

Future efforts to improve separation of the two envelope membranes will have to address the issue of how to disrupt the contact sites. With rat heart mitochondria, an effort has been made to disrupt contact sites by including proteolytic enzymes in the buffers used for mitochondrial fractionation [34]. Alternatively it may be possible to disrupt contact sites by appropriate chemical treatments.

PROPERTIES AND FUNCTIONS OF THE DOUBLE MEMBRANES

If one examines the properties of the membranes from the three systems, several conclusions can be made. The first is that in each system, the outer membrane differs from the inner (Table II). For example, in each system the two membranes differ significantly in buoyant density, reflecting their different chemical compositions. Second, the comparable membranes from the three systems have some important differences among them. For example, the inner membranes of mitochondria and bacteria contain the components of respiratory electron transport and oxidative phosphorylation, whereas the chloroplast inner envelope membrane does not. Despite these differences, interesting functional similarities exist among the three systems. In this review, we choose to discuss three such similar functions: (1) the transport of ions and metabolites by the inner membrane; (2) the translocation of polar lipids between membranes; and (3) the transport of proteins into and across the membranes.

Transport of Ions and Metabolites

Chloroplasts, mitochondria, and Gram-negative bacteria are metabolically very complex systems that require the transport of a variety of metabolites across their

double-membrane envelopes. Chloroplasts and mitochondria export large amounts of photosynthetic products and citric acid cycle intermediates, respectively, into the cytoplasm and can import or export ATP, phosphate, and amino acids depending on the organelle's metabolic state. These two organelles interact with a common cytoplasmic pool of metabolites and have some similar translocator requirements, eg, the need to transport ADP and phosphate for photophosphorylation or oxidative phosphorylation and to transport amino acids for protein synthesis. Thus, these organelles might be expected to have some common mechanism for metabolite transport. Bacteria, on the other hand, experiences a more uncertain environment and have little need to export nucleotides, amino acids, or other metabolites. For this reason, one might expect bacteria to have different mechanisms for transport than do mitochondria and chloroplasts. Accordingly, only the specific carriers of mitochondria and chloroplasts will be discussed.

The few carriers that are known to exist in chloroplasts have been identified and characterized primarily in intact chloroplasts [1]. The best-characterized chloroplast translocators are the phosphate carrier, the dicarboxylate carrier, and the ATP/ADP carrier [1]. Of these only the phosphate carrier has been identified [35], partially purified [36], and reconstituted into liposomes [36]. In mitochondria both a phosphate translocator and an ATP/ADP exchange carrier have been extensively studied [37]. Thus, for at least two of the metabolite carriers in chloroplasts there appear to be homologous carriers in mitochondria. The chloroplast dicarboxylate translocator has an extremely broad specificity based on substrate competition experiments [38]. Although it has been suggested that the dicarboxylate translocator may be composed of several carriers [38,39], this has not been demonstrated. In mitochondria no fewer than six carriers have been reported to carry metabolites, which are thought, in chloroplasts, to be transported by a single dicarboxylate carrier [17]. Because both the mitochondria and the chloroplast have several different metabolic processes which involve these dicarboxylates, it seems likely that the chloroplast may also have a diversity of dicarboxylate carriers.

In terms of energetics, mitochondria have been found to have four types of transport. These include (1) electrogenic transport which is sensitive to $\Delta\psi$; (2) proton compensated electroneutral transporters which are sensitive to ΔpH; (3) electroneutral anion counter-exchange; and (4) neutral metabolite transport [37]. At present, the chloroplast carriers whose energetics have been studied are reported to mediate counter-exchange [1]. Only the uptake of glycerate has been reported to be sensitive to light and uncouplers [40].

From these comparisons it seems unlikely that our current, simplified models of metabolite transport across the chloroplast envelope will prove adequate. It seems reasonable to expect that, as more is known about metabolite transport in chloroplasts, the carrier systems will be at least as complex as those of mitochondria.

Translocation of Polar Lipids

The double membranes in all three systems are the sites of polar lipid synthesis. In bacteria, the inner membrane is the site of synthesis of both lipopolysaccharide and phospholipids [41,42]. In chloroplasts and mitochondria the details of the biosynthetic process are still being elucidated, but it appears that both inner and outer membranes participate in lipid synthesis [43–45]. For example, in pea chloroplasts, there is evidence that the final assembly of galactolipids occurs on the outer envelope mem-

brane [31], whereas diacylglycerol is synthesized in the inner envelope membrane [29,46; J. Andrews, unpublished observations]. What is clear is that, with the exception of the bacterial inner membrane, none of the membranes in the three different systems synthesize all of their constituent polar lipids [43–45]. Thus, lipids must be shuttled between membranes. The problem is even more complex in chloroplasts. Galactolipids must be distributed between the envelope membranes, and must also be transported from the inner envelope membrane across the soluble stromal space and incorporated into the thylakoids [44,47].

The lipid transfer process has been best studied in bacteria where both lipopolysacchharide and phospholipids must be transferred from inner to outer membrane [41,42]. It has been shown that the transfer process is very rapid. For example, newly synthesized phospholipids migrate from the inner membrane to the outer membrane with half lives of 0.5–3.0 min [48]. This transfer is also bidirectional; phospholipids can move from outer to inner membrane as well as the other direction [49,50]. At present, the mechanism of such transfer has not been established. A number of mechanisms are possible. Conceivably, soluble carrier proteins could mediate the flow of lipids between the membranes. However, this mechanism seems unlikely in view of the relatively non-specific nature of the transfer process. Foreign lipids not normally present in bacterial membranes can be transferred from one membrane to another [49,50]. For this and other reasons, it has been suggested that lipid transfer is mediated via the contact sites where the two membranes are held in close proximity [49,50]. In support of this model is the observation that newly synthesized lipopolysaccharide appears on the outer membrane in multiple discreet locations [41]. These locations appear to be preferentially positioned over membrane adhesion sites [41]. However, recent studies on the transport of lipopolysaccharide indicate the process may be more complex than originally envisioned [51], and more extensive studies need to be undertaken.

In chloroplasts, virtually nothing is known about the lipid transfer process. In fact, we are only now learning which lipids must be transferred between membranes. Nevertheless, studies of lipid transport among chloroplast membranes promises to be an intense area of future research. Chloroplast membranes can account for up to 75% of the total cellular membrane in leaf tissue [52]. Thus, the assembly of their polar lipids is a major cellular process which deserves to be elucidated.

Transport of Proteins Into and Across Membranes

The manner in which newly synthesized proteins are inserted into or translocated across membranes is currently of intense scientific interest [3,53–61]. Much of what we know about these processes derives from studies of the transfer of proteins into and across the endoplasmic reticulum membrane [54,55,57]. Proteins also need to be transported into and across the double membrane systems of mitochondria, chloroplasts and Gram-negative bacteria [3,56–61]. In bacteria, protein synthesis occurs in the cytoplasm. Some of these proteins cross the inner membrane and are soluble in the periplasm; others cross the inner membrane and are inserted into the outer membrane; still others cross both membranes and are secreted [58]. Proteins in chloroplasts and mitochondria are synthesized not only inside the organelle, but also outside in the cytoplasm of the cell [3,62]. These cytoplasmically synthesized proteins must be transported into the organelle. Mitochondrial proteins must cross one or two membranes in order to achieve their final location. With chloroplasts, the process

appears to be even more complex. In addition to the two envelope membranes and the soluble compartments which they enclose, an additional membrane-bounded compartment within the chloroplast is defined by the thylakoids. A number of thylakoid membrane proteins, and at least one protein found in the lumen of the thylakoids, are synthesized in the cytoplasm [62–64]. In this latter extreme case, in order to arrive at its final location the protein must in effect cross three membranes and two soluble compartments.

Despite some obvious differences among the three systems, some important similarities also exist. In all three systems, an additional amino terminal signal peptide is required for proteins that are to be transported across at least one membrane [56–64]. In bacteria and mitochondria, a signal peptide does not appear to be required for proteins that are inserted into the first membrane [58,65–67]. For bacteria this is the inner membrane; for mitochondria it is the outer membrane. At present, the biogenesis of chloroplast outer membrane proteins has not been studied.

Another similarity is that all three systems have an energy requirement for transport [68–72]. The energy source in mitochondria and Gram-negative bacteria is a membrane potential [68–70]. Originally it was reported that mitochondrial protein transport required ATP rather than a membrane potential [71]. However, subsequent work demonstrated the requirement for a membrane potential [70]. In chloroplasts, Grossman et al [72] have reported that a membrane potential is not required, but that ATP is necessary for protein transport.

Much effort is presently being devoted to understanding the functions of both the signal peptide and the energy requirements for transport. However, the solutions to these problems are not clear in any of these systems. For instance, it is not known whether the membrane potential is the driving force for translocation of the proteins or simply a catalyst which alters membrane structure [59]. In this regard it should be noted that proteins to be secreted across bacterial membranes experience a trans-positive membrane potential whereas proteins to be secreted into mitochondria experience a trans-negative potential [59,60].

Finally, little is known about the intermediate steps involved in crossing two bilayers. It has been suggested that in all three systems, proteins which cross both of the bounding membranes are transported at the contact sites [59,60]. In bacteria and mitochondria there is some suggestive evidence that this is the case. For example, it has been found that 80S ribosomes copurify with yeast mitochondria [73]. Electron microscopy of the isolated mitochondria show that the 80S ribosomes are bound to the outer mitochondrial membrane at the sites of membrane attachment [73]. However, in all three systems we are still far from understanding how protein transport occurs. A better understanding of each membrane and the manner in which the two membranes interact will be important in gaining this understanding.

SUMMARY

In this review we have attempted to briefly compare the double membranes of chloroplast envelopes, mitochondria, and Gram-negative bacteria and to discuss the homologies among the three systems. Although there are some obvious differences, strong similarities are also apparent, both at the structural and at the functional levels. Structurally, all three systems share the property of having outer membranes that are permeable to low molecular weight compounds and inner membranes that are im-

permeable except via specific translocators. Another common structural feature is the existence of sites of adhesion (contact sites) between inner and outer membranes. In a practical sense, these contact sites appear to present problems in purifying the individual membranes. Cross contamination of membranes is a problem in all three systems. With the chloroplast envelope, we have obtained evidence that the sites of attachment lead, at least in part, to vesicles of inner and outer membrane that are physically intermingled. Future refinements in membrane purification procedures will likely focus on nondestructive methods for disrupting these areas of membrane attachment.

We have discussed three similar functions of the membrane systems. With respect to metabolite transport by the inner membrane, it appears that mitochondria and chloroplasts are more homologous to each other than either is to Gram-negative bacteria. Currently, studies on transport into chloroplasts portray a relatively simple process involving only a few broad-specificity carriers, whereas studies on mitochondrial metabolite transport indicate a much more complex process involving multiple carriers and sophisticated energetics. Because mitochondrial transport is the much more thoroughly studied and characterized system, the findings with mitochondria may provide insights into future research on transport across the chloroplast envelope.

Finally, chloroplasts, mitochondria, and Gram-negative bacteria have been compared with respect to transfer of lipids between membranes and transfer of proteins across membranes. The mechanism involved in both of these processes have as yet not been worked out. Yet some interesting similarities appear to exist among the three double-membrane systems. The contact sites may play a role in mediating interactions between membranes during the transport process. If this is so, then advances in one system may be also applicable to studies of protein and lipid transport carried out in the other two systems.

The similarities among the double-membrane systems of Gram-negative bacteria, mitochondria, and chloroplasts may be explained in part by the endo-symbiotic theory for the origin of the organelles [74]. It may be that all three are derived from some common ancestor. This ancestor could have evolved to modern free-living bacteria and also given rise to the endo-symbionts that became mitochondria and chloroplasts. Although it is not understood at present, there may be some selective advantage to retention of the double-membrane envelope in all three systems.

We hope this comparative review will stimulate an increased awareness of the importance of the chloroplast envelope membranes and the potential they offer for future investigations.

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