9

ENERGY-ORIENTED ORGANELLES AND ACTIVITIES: II

The Mitochondrion

Among eukaryotes the cyclic respiratory processes just described occur for the greater part in an organelle of the cytoplasm known as the mitochondrion, while glycolysis and related activities are confined to the cytoplasm. In the prokaryotes mitochondria are absent, but many types of those organisms possess a membranous body in which the citric acid cycle may proceed. Because the eukaryotic organelle has been far more extensively explored, its structure receives attention prior to the simpler bodies found in bacteria and their relatives. Although the primary function of the mitochondrion is in cell respiration, it seems to be involved in numerous other aspects of the cell's economy, as attested by the differing enzyme systems found from tissue to tissue. Most of these roles remain unknown, but some are coming to light at the current time. For instance, in earthworm spermiogenesis it plays an evident part in the condensation of the chromatin in the nucleus (Figure 9.1; Martinucci and Felluga, 1979). Moreover, it has been found to be active in mediating the action of luteinizing hormone in the synthesis of steroids in Amphibia (Wiebe, 1972).

9.1. MITOCHONDRIAL STRUCTURE

As a consequence of both the relative ease with which its fine morphology is revealed and the innumerable experiments that have elucidated its major functions, the mitochondrion doubtlessly ranks among the best known organelles of the cell. Yet, well explored as it is, much about it still remains either entirely unknown or subject to strongly conflicting interpretations. Furthermore, the vast majority of electron microscopic and experimental investigations have been made upon the mitochondrion of but a single group of organisms,

L. S. Dillon, *Ultrastructure, Macromolecules, and Evolution*

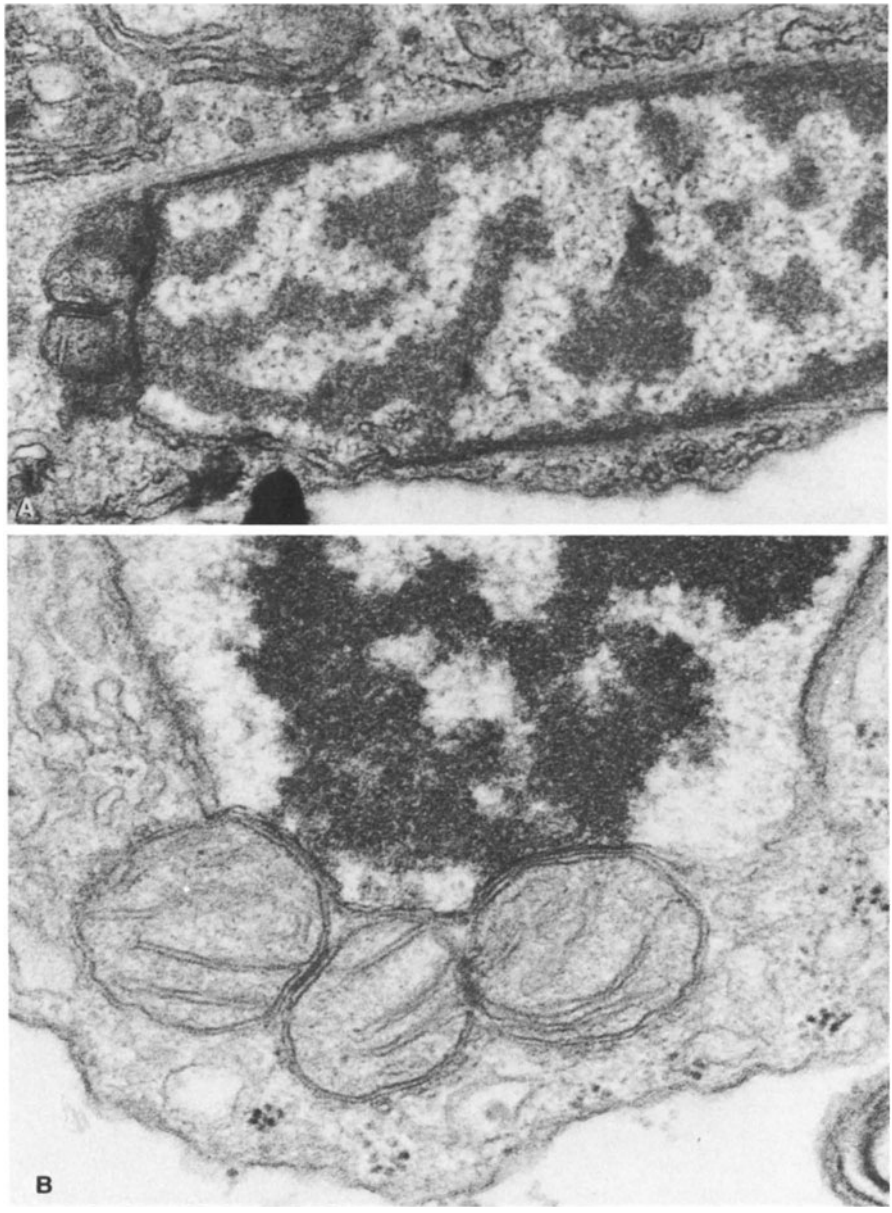


Figure 9.1. A role for the mitochondrion during spermiogenesis. During spermiogenesis in lumbricoid annelids, the mitochondria play a major role in condensing the chromatin into bodies called prochromosomes. (A) A number of electron-opaque prochromosomes appear in the nucleus. 20,000×. (B) In this enlarged view of the end of the foregoing, the relationship between condensing chromatin and three mitochondria is made clear. 63,000×. (Both courtesy of Martinucci and Felluga, 1979.)

the metazoans—or more specifically, the vertebrates. Because of this concentration of knowledge within a single area, it is advantageous first to discuss the organelle found among those animals, even though it be an advanced type, and then to make comparisons with the others later.

9.1.1. The Mitochondrion of the Metazoa

The Standard Structure. So familiar is the standard ultrastructure of the metazoan mitochondrion that it is almost superfluous to describe it here (Steinert, 1969). According to the familiar interpretations of the morphology, the organelle is enclosed by two parallel, adjacent membranes, the narrow space between being the outer compartment (Whittaker, 1966). The large inner compartment (Figure 9.2A) is filled with a more or less homogeneous ground substance, usually called the stroma or matrix. Across the stroma extends a number of cristae, membranous structures that typically are flattened sacs, usually thought to be formed by invagination of the inner enveloping membrane. Within the lumen of each crista (the intracristal space) is a limited quantity of material that typically is somewhat more electron transparent than the stroma itself (Figure 9.2A).

The familiar view of the organelle is currently undergoing a somewhat radical change in some of its features as a result of improved techniques. First, it was shown that in mitochondria from tissue that was frozen within a few sec-

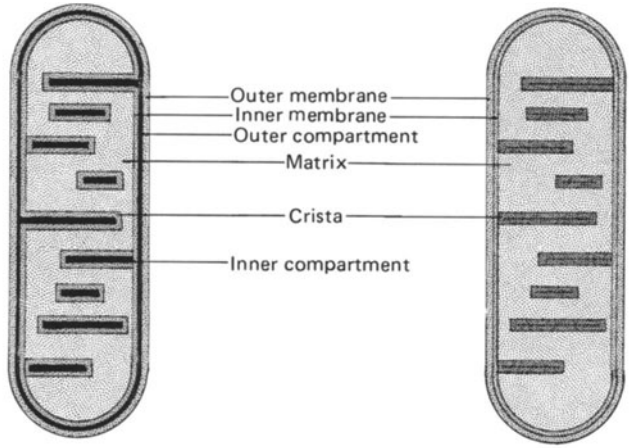


Figure 9.2. Two views of standard mitochondrial structure. Techniques currently being developed suggest that neither an outer compartment nor the intracristal space actually exists (B), as they do when prepared by standard techniques (A).

onds after death, the two sides of the cristae were appressed, so that they formed a single double membrane without an intracristal space separating them (Malhotra, 1966). Moreover, with this treatment, no outer compartment was present (Figure 9.2B). Avoiding use of osmium tetroxide, which has a deteriorating effect on membranes, and combining freeze-drying with low-temperature embedding in plastic, Sjöstrand and his co-workers confirmed and extended these observations (Sjöstrand and Kretzer, 1975; Sjöstrand and Bernhard, 1976; Sjöstrand, 1977, 1978; Sjöstrand and Cassell, 1978). Thus it appears that in living material, the mitochondrion consists simply of a dual-membrane enclosure containing a matrix-filled inner compartment that is traversed by a number of flat double-membraned structures called cristae (Figure 9.3B).

Although the major ultrastructural characteristics are remarkably consistent throughout the Metazoa, variations upon the general theme are often encountered. For example, the number of cristae per mitochondrion varies from one tissue to another, being high in striated muscle and low in kidney and liver cells. Sometimes, as in kidney cells of overwintering frogs, the cristae parallel the longitudinal axis of the mitochondrion, whereas those of active summer frogs are of typical construction. In the inactive frogs, the longitudinal orientation is associated with low cytochrome oxidase activity (Karnovsky, 1963). A further example of this variation is found in the mature eggs of *Tubifex*, a freshwater annelid, and still others have been reported from pineal cells of the rat (Lin, 1965) and human uterine mucosa cells during the midmenstrual period (Merker *et al.*, 1968). In addition to a frequent elongate condition, the mitochondria of the pineal cells were often filled with a number of fiber-containing cylinders that replaced most of the central cristae.

Modifications in Chloride Cells. In the gills of a number of marine organisms, devices for eliminating salt from the body, or conversely, absorbing it from the sea, have been developed that involve the mitochondrion, often highly modified for the function. In certain fish, including *Fundulus heteroclitus* (Philpott and Copeland, 1963), the mitochondria have the cristae oriented longitudinally, not unlike the arrangement just described in hibernating frogs. Moreover, a second more diversified type is present, but its discussion needs to be reserved for a later section. Still different mitochondrial modifications exist in the sea lamprey, in which this organelle is associated with a proliferation of microtubules (Figure 9.4A; Peek and Youson, 1979).

A greatly modified variant of the cristate mitochondrion, however, has been described from salt cells of the blue crab (*Callinectes sapidus*). In this arthropod these specialized cells are most frequently employed for absorbing salt from the medium when the animal is living under low saline conditions (Copeland and Fitzjarrell, 1968), but they can also serve in the opposite capacity and secrete salt during high salinity periods (Mantel, 1967). While the usual form of mitochondrion is also present, specialized ones are arranged singly within the numerous, close-set pinocytotic folds of the plasmalemma. This

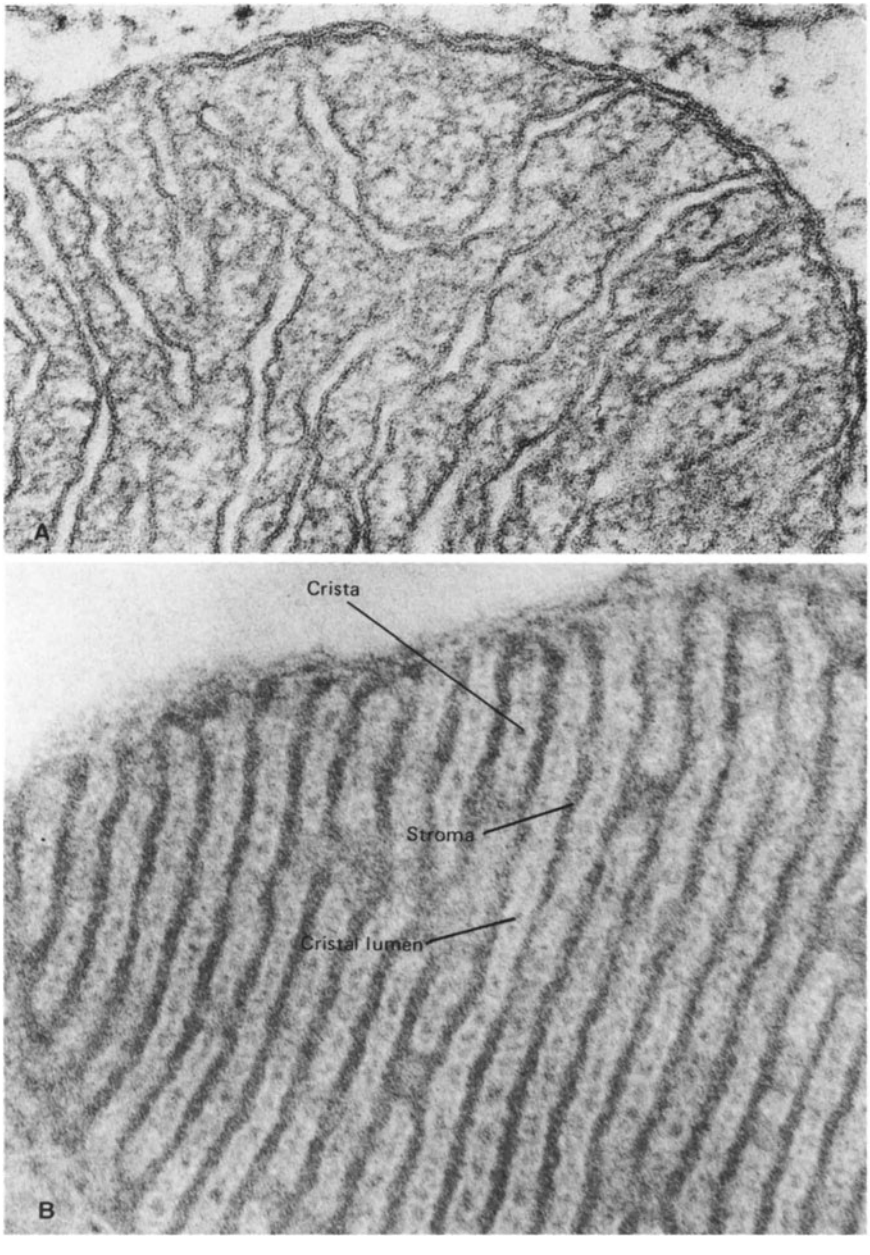


Figure 9.3. Ultrastructure of the mammalian mitochondrion. (A) Rat heart mitochondrion prepared by standard techniques shows much stroma and distinct intracristal lumina. 120,000×. (B) Same but prepared by modified techniques. The stroma is confined to narrow interspaces between the compressed cristae. 180,000×. (Both courtesy of Sjöstrand, 1977.)

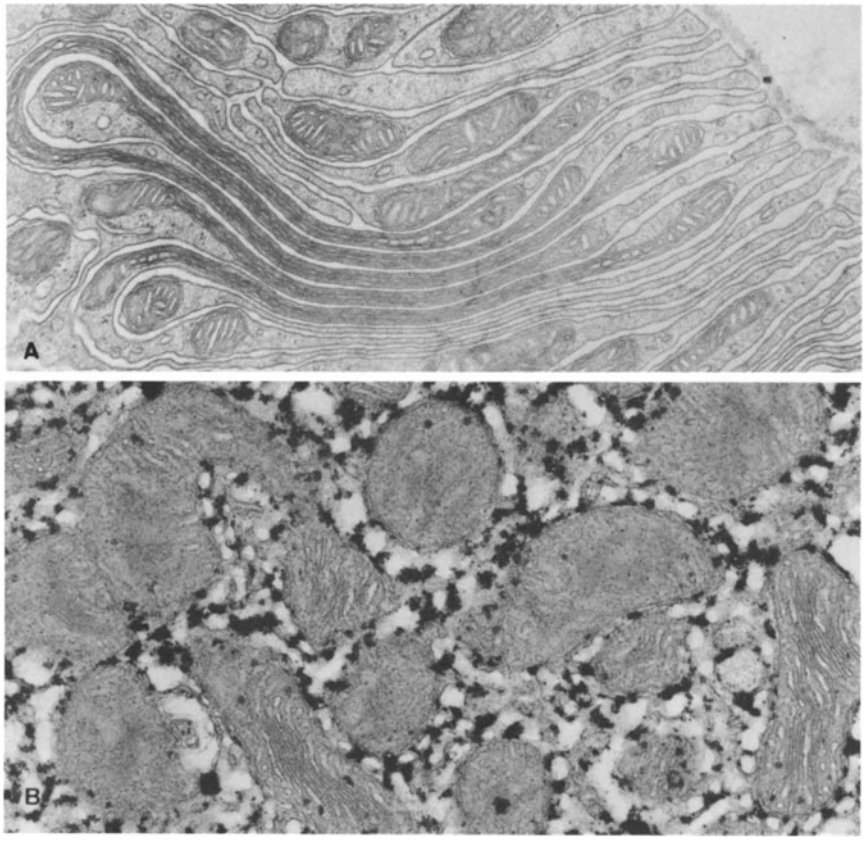


Figure 9.4. Modifications of the metazoan mitochondrion in salt cells. (A) Cup-shaped mitochondria in the salt cells of the blue crab. 17,000×. (Courtesy of Copeland and Fitzjarrell, 1968.) (B) Section of epithelium of the chloride cells of the pinfish, treated to show sites of $Na^+ + K^+$-ATPase activity, most of which is localized in the plasmalemma labyrinth rather than in the mitochondria. 26,000×. (Courtesy of Hootman and Philpott, 1979.)

modified type is greatly elongate and has the periphery swollen; often it is discoidal but frequently may be infolded into a cup-shaped configuration (Figure 9.4A). In the prothoracic gland of fourth-instar larval silkworms, the mitochondria are similarly elongate and cup shaped, with a single crista, but the enlarged peripheral portion is vesicular rather than cristate (Beaulaton, 1968). Since most of the $Na^+ + K^+$ ATPase activity has been shown to be located in plasmalemma folds (Figure 9.4B), the mitochondrion may simply supply the necessary energy (Hootman and Philpott, 1979).

The Microvillose Pattern. In several instances among the Metazoa, the

organelle displays a morphology so strikingly different that it is precluded from being considered a mere variant of the familiar type. Instead of flat disks, numerous tubular cristae, often called microvilli, penetrate the stroma from all sides (Figures 9.5, 9.12B). Among reptiles, birds, and mammals, this microvillose variety appears mainly to characterize cells of the adrenal cortex, but it also has been reported from the tail muscle cells of larval *Xenopus*. Because most of the details of structure somewhat resemble those of the organelle in many ciliated and amoeboid protozoans and other protistans discussed later, it appears to be more primitive than the cristate form. Unfortunately, almost no attention has been given to the finer particulars of its structure, function, or molecular organization.

A modification of the foregoing, although occurring most frequently in such active cells as those of skeletal and cardiac muscle, has been observed also in numerous other tissues and in a great variety of metazoans. In this variant, the microvilli are bent sharply several times into a zigzag pattern. As a rule they are more highly ordered than in the commoner microvillose type, forming several rows down the length of the organelle, with the denticles of each row dovetailing into the adjacent ones. Some chloride cells from gills of *Fundulus heteroclitus* have this pattern, not the longitudinal cristae noted above; the same cells from the skin of a very young sardine (*Sardinops caerulae*) have a similar structure (Lasker and Threadgold, 1968). Other examples of this type among metazoans include gastric mucosal gland cells of the frog and the right ventricular papillary muscle of the cat.

Another variation of the microvillose type has been reported from the vinegar eelworm (Zuckerman *et al.*, 1973). Although most of the mitochondria were of the normal flat cristate type, an occasional large one was encountered in which the cristae were tubular, with greatly thickened membranes (Figure 9.5). In addition, a variable number of paracrystalline arrays were present. Still another variant of the microvillose type occurs in a number of metazoans, including such diversified subjects as bats (pancreas and cricothyroid muscles), grass frog (gastric mucosa), salamander (liver), and the medicinal leech (nervous tissue) (Revel *et al.*, 1963). In addition, it is found with the other types mentioned in preceding paragraphs as occurring in the gill chloride cells of *Fundulus*. The outstanding characteristic of this variety is the prismatic form of the cristae, which are triangular in cross section. As a rule, the prismatic tubules are located amid others of the usual type, often forming a central bundle as illustrated (Figure 9.6).

Differences between metazoan mitochondria are not confined to structural features but also are being demonstrated at the functional level, as exemplified by a study on the rat liver cell (van Berkel and Kruijt, 1977). In this mammal, about 65% of the liver mass consisted of parenchymal cells (hepatocytes) and about 10% nonparenchymal. Isolation of pure fractions of intact cells of each type permitted the demonstration of distinct differences in the activities of the

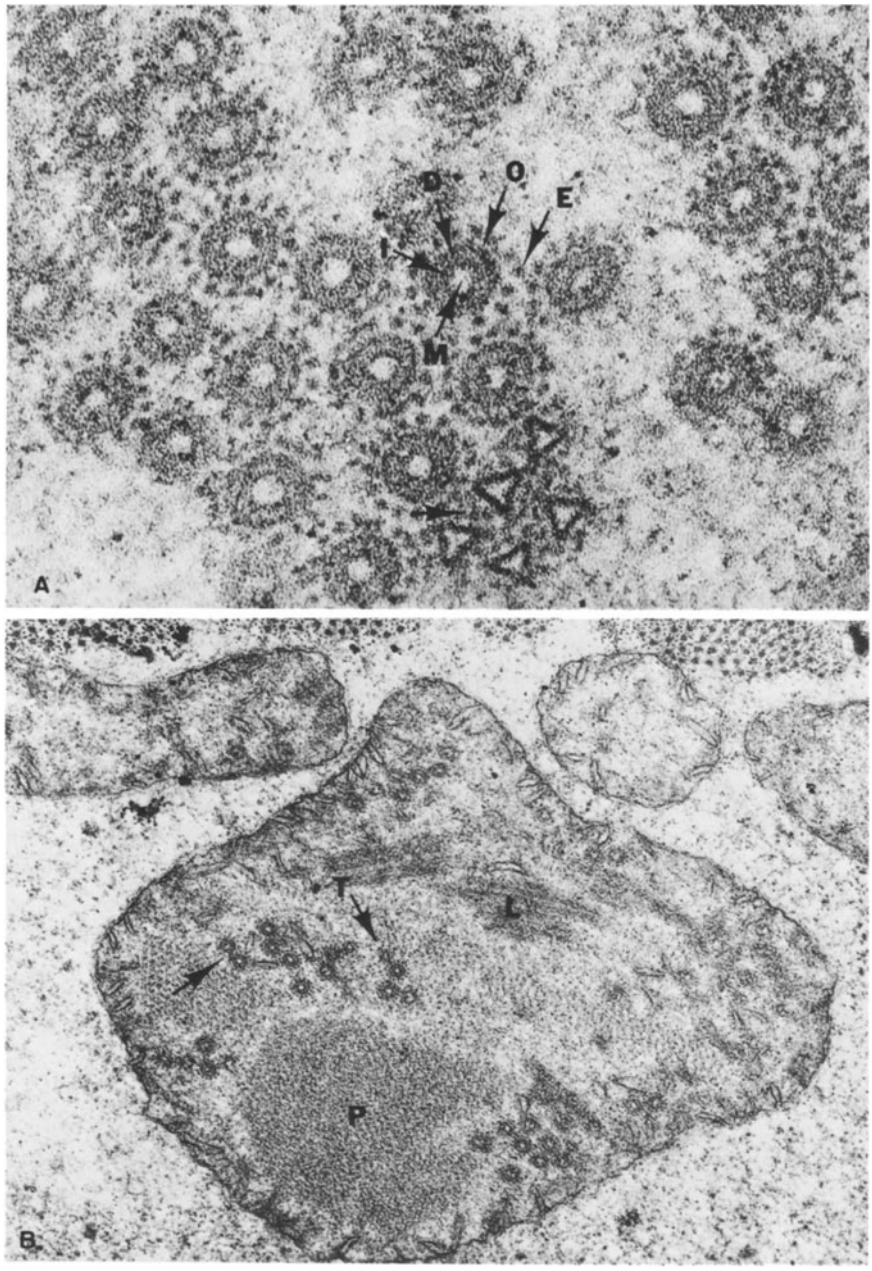


Figure 9.5. Unusual cristae in a microvillose mitochondrion. (A) The mitochondrion of the vinegar eel (*Turbatrix aceti*) consists of short microvilli around the periphery, longer modified ones centrally, and paracrystalline arrays. E, capitate particles; D, matrix; O, outer membrane; I, inner membrane; M, lumen. 33,000×. (B) The modified cristae are doughnut shaped and bear capitate particles over their surface; a few that are triangular in cross section are also evident. P, paracrystalline arrays; L, longitudinal section; T, transverse section. 150,000×. (Both courtesy of Zuckerman *et al.*, 1973.)

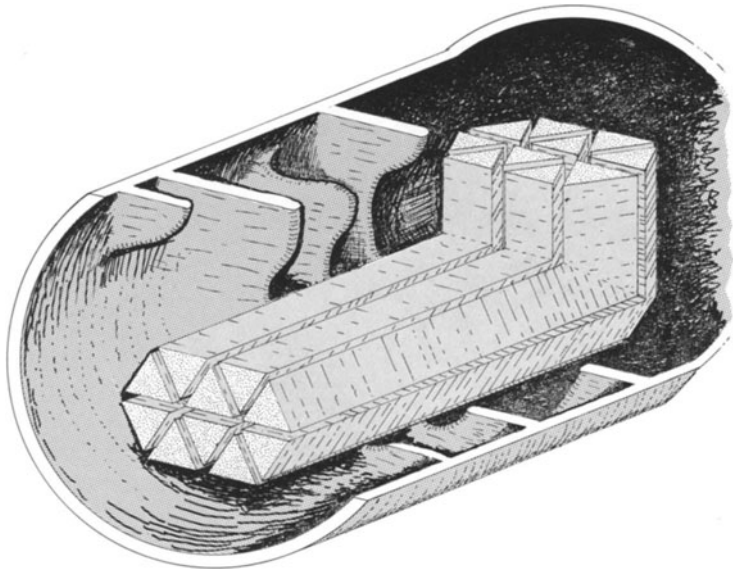


Figure 9.6. A prismatic variation of the microvillose mitochondrion. (Based on Revel *et al.*, 1963.)

mitochondrial enzymes. Except for monoamine oxidase, which was equally active in both cell types, the specific activities were generally lower in nonparenchymal cells. Pyruvic acid carboxylase activity was only 2% that of the parenchyma, glutamic acid dehydrogenase 4.3%, and cytochrome *c* oxidase 79.4%. Moreover, only one form of the pyruvic acid carboxylase was present, instead of the two of the hepatocytes.

Physiological-State Effects. Recent studies indicate that the actual morphological state of any given mitochondrion is associated in large measure with physiological conditions (Green *et al.*, 1968; Penniston *et al.*, 1968; Harris *et al.*, 1969; Cieciura *et al.*, 1978, 1979). A "nonenergized" (orthodox) pattern, in which the cristae are flat disks as in the typical metazoan organelle, is seen in electron micrographs of rat heart mitochondria prepared by the usual techniques. Similar configurations are obtained by use of 2,4-dinitrophenol, a reagent that discharges the energized state of the membranes. Under energizing conditions, such as those induced by the presence of a 10 mM concentration of sodium succinate, the cristae become swollen (condensed or energized) or swollen and zigzagged (energized-twisted) (Hackenbrock, 1966, 1968). Mitochondria from other tissues (Figure 9.7), including rat liver and kidney, ox retina, and canary flight muscle, react in identical fashion (Vail and Riley, 1972; Grimwood and Wagner, 1976). Similar changes have been noted to occur in the eggs of sea urchins. In the unfertilized egg of these animals, the

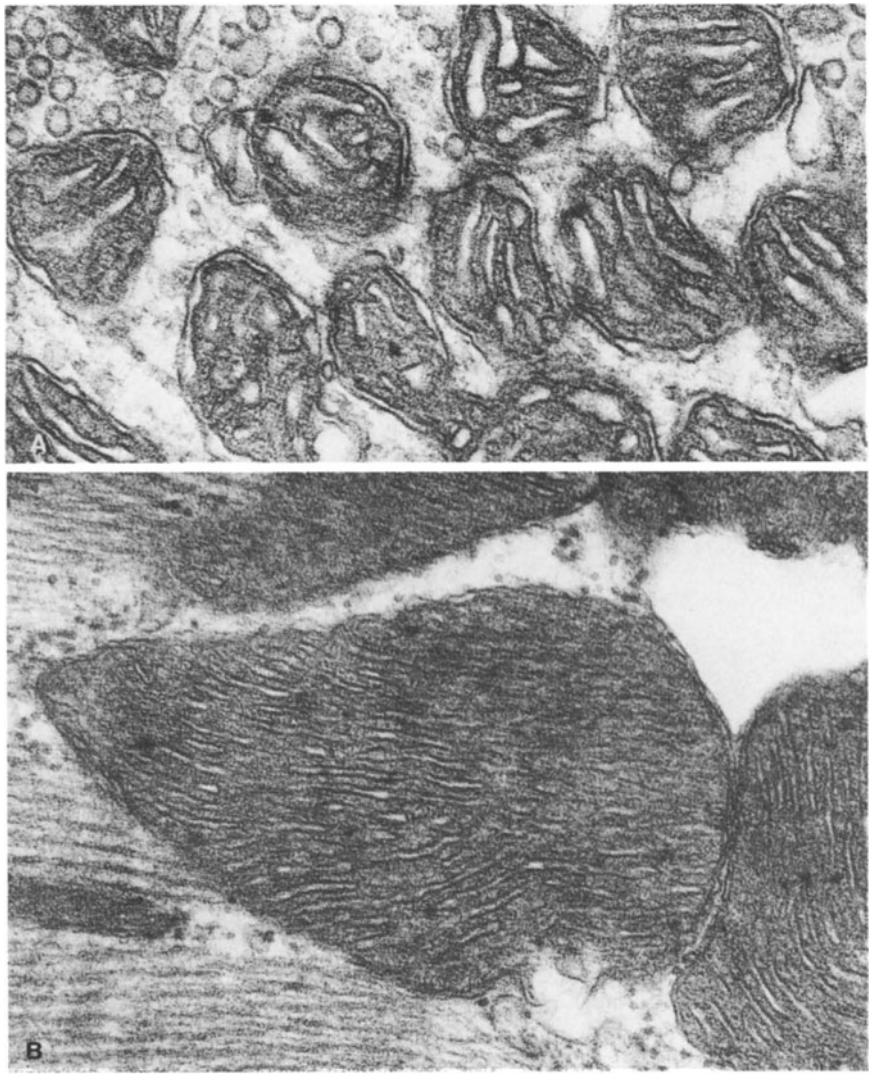


Figure 9.7. Changes in morphology with the physiological state of the cell. (A) Mitochondria of a mossy fiber of the granular layer of the rat cerebellum approach the condensed, or energized, state. 67,500×. (B) Mitochondria of rat heart muscle cell. 67,500×. (Both courtesy of Cieciura *et al.*, 1979.)

mitochondria were found to be in the condensed (energized) configuration, but all were in the orthodox form after fertilization had occurred (Innis *et al.*, 1976).

Effects of the physiological state of the organism as a whole have also been reported on numerous occasions, several of which, such as overwintering frogs, have already received mention. Hibernation in rodents, such as the dor-

mouse, induced alterations in mitochondria of the kidney tubule cells (Amon *et al.*, 1967). These organelles accumulated ferritin as the cristae degenerated and the matrix became more electron opaque. Near-freezing temperatures in the environment altered both the size and the number of cristae in rats, the organelle of brown adipose tissue becoming considerably enlarged and the cristae more numerous (Suter, 1969). These changes were reversed when the animals were returned to warm temperatures for a week or more. Moreover, prolonged starvation in the Japanese newt led to the development of three types of inclusions (Figure 9.8; Taira, 1979).

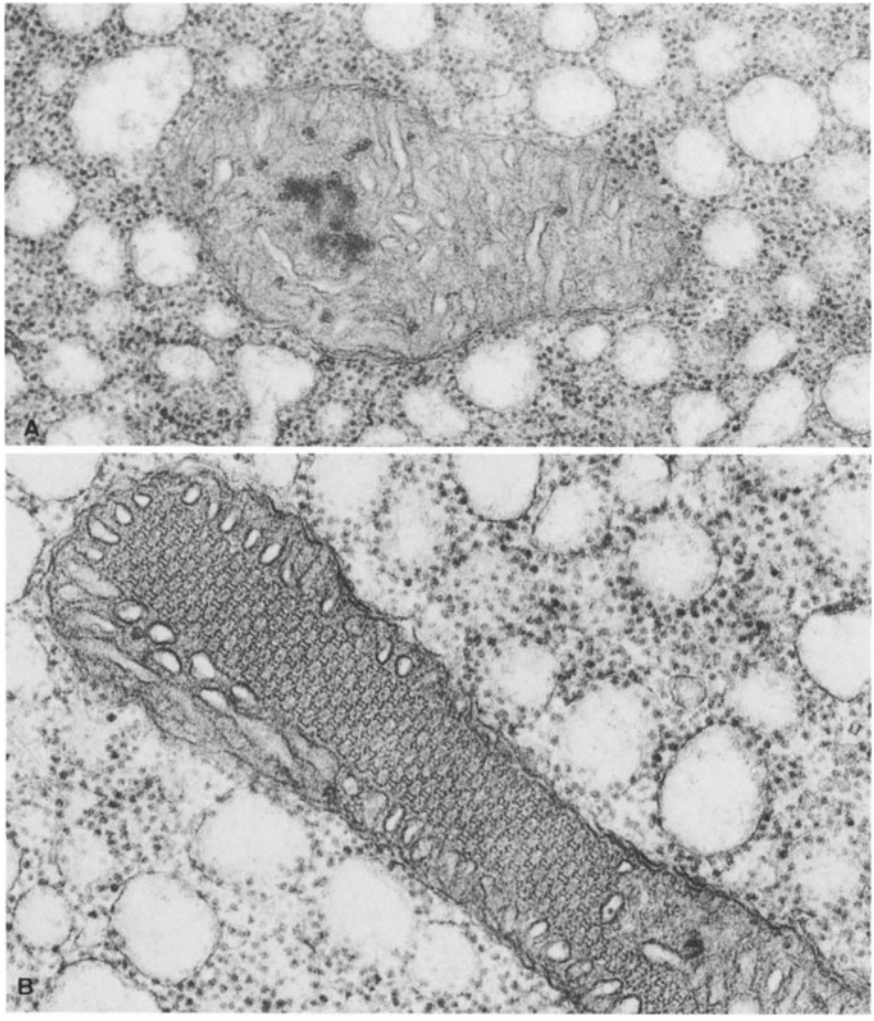


Figure 9.8. Alterations in mitochondrial structure induced by starvation. Three types of inclusions were found in mitochondria of Japanese newt cells after 1 week of starvation, including electron-opaque bodies (A) and crystalloids (B). (A) 43,000×; (B) 52,000×. (Both courtesy of Taira, 1979.)

One case involving reproductive physiological events has already been enumerated, but many others are known. For instance, in the females of the genus *Locusta*, the mitochondria of the corpora allata became greatly enlarged as sexual maturation proceeded. With this enlargement, the cristae elongated and became hooplike, while the matrix increased in electron density (Fain-Maurel and Cassier, 1969). When the ovaries commenced to ripen, the second type of this organelle developed, having a dumbbell shape and longitudinal cristae. Moreover, morphological and physiological changes have been noted in the germ cell mitochondria of rats (DeMartino *et al.*, 1979). In secondary spermatocytes and again in spermatids, those organelles assumed a rounded configuration and the condensed arrangement of the cristae. At the same time, their respiratory control ratio was very high, suggesting that they were functionally very active.

9.1.2. The Mitochondrion of Higher Plants

Although the mitochondrion of the Metaphyta has received considerable attention (Hackett, 1959; Diers and Schötz, 1965; Parsons *et al.*, 1965), it has not been studied nearly so intensively as that of the metazoans. Perhaps some of the relative neglect of the green plant organelle results from the greater difficulties associated with its isolation and preparation, or perhaps it stems in measure from the greater attention focused by plant cytologists upon that unique organelle of their subjects, the chloroplast. Nevertheless, enough has been learned that the major features of its structure and biochemistry may be outlined.

Structure. The mitochondrion of higher plants is generally considered similar in structure to the metazoan organelle (DeRobertis *et al.*, 1970); close comparison, however, discloses a number of small but persistent differences. By and large, in the plant there is a far higher percentage of stroma present, with the cristae few in number and tending to be irregular, both in form and disposition (Figure 9.9). Frequently, the plane in which the expanded portion of the cristae is oriented varies widely, so that in a single surface exposed by sectioning, some of these structures are cut longitudinally and others transversely. As microvillose cristae often are interspersed among flattened discoidal ones, electron micrographs of the higher plant mitochondrion reveal a strange mixture in the stroma, consisting of cylinders sectioned at various angles, ghostlike images of membranes cut parallel to their surfaces, and the familiar partitionlike formations of metazoans. Moreover, the outer membrane appears to be completely covered by minute pits; by actual measurement in white potato mitochondria, the pits were found to be from 25 to 30 Å in diameter and spaced at about 45-Å intervals, center to center (Parsons *et al.*, 1965).

In mesophyll cells and others actively engaged in photosynthesis, the cristae of the mitochondria are more close set and tend to be relatively short;

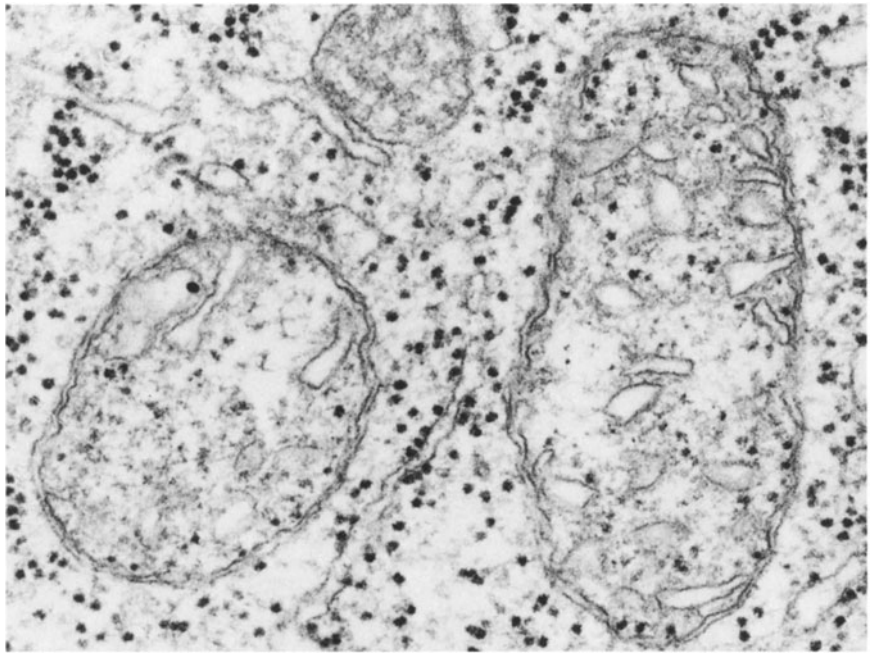


Figure 9.9. The metaphytan mitochondrion. The mitochondrion of higher plants contains a mixture of inflated saclike and tubular cristae, sparsely placed, as in this root tip cell of corn. 40,000×. (Courtesy of Hilton H. Mollenhauer, unpublished.)

frequently, they merely fringe the periphery, leaving a central area free. The few studies by freeze-etch techniques (e.g., Branton and Moor, 1964) have thus far contributed little to further understanding of the mitochondrion's morphology but confirm the observations made by standard procedures.

9.1.3. The Mitochondrion of Other Eukaryotes

Among the remainder of the eukaryotes, mitochondrial features distinctive for the respective taxa are disappointingly meager. In broad terms, the amoeboid and ciliated protozoans possess mitochondria with microvillose cristae, a condition that is also catholic among most of the eukaryotic algal groups, including both chlorophyllaceous and colorless flagellates. Only the green algae are exceptional to some extent, in that the organelle, not surprisingly, more closely approximates that of the metaphytans (Lang, 1963; Lembi and Lang, 1965; Lloyd and Venables, 1967). Among important variants known to exist are the several that follow.

Mitochondria of Fungi. In general, the mitochondria of fungi are like those of other protistans in being of the microvillose type (Figure 9.10), the microvilli being rather coarse and greatly distended, as in *Synchytrium* (Lange and Olson, 1978). Moreover, they are relatively long, usually half the width of the inner chamber and sometimes nearly fully as long. How many of these traits would alter with the use of more recent techniques remains for the future to disclose. In some cases the overall appearance differs from the preceding description, the microvilli being fine in cross section, relatively sparse, and shorter than half the width of the inner chamber. In addition, there is a Feulgen-positive central body (Kuroiwa *et al.*, 1976a,b), which can be stained with thionine for viewing by light microscopy. Under the electron microscope, it is seen as an electron-dense cylinder, oriented with the long axis of the mitochondrion (Kuroiwa *et al.*, 1977). This body has been shown to contain a large fraction of the DNA present in the organelle and has been observed to undergo division in unison with the rest of the structure (Kuroiwa, 1974). During these processes, the central cylinder, called the nucleoid, elongates as the entire organelle does likewise; then after a cleavage furrow has formed around the equatorial region of the mitochondrion and has eventually deepened, it becomes divided into two, much as in the bacterial nucleoid (Chapter 11). The close affinity to the other fungi of the so-called protozoan taxon Mycetozoa is clearly indicated by the presence of this unique feature in their mitochondria, as shown by *Didymium* (Schuster, 1965). Among botanists this genus is already classed as a slime mold.

In addition, bodies somewhat resembling the mesosome of bacteria, described later in this chapter, have been reported for various lower fungi (Zachariah and Fitz-James, 1967; Malhotra, 1968; Kozar and Weijer, 1969). However, examination of the published electron micrographs discloses that most of these structures are located within vacuoles and in some cases appear to be degenerating mitochondria. Others possibly are artifacts, as has been suggested (Curgy, 1968).

The Euglenoid Mitochondrion. At first glance the mitochondrion of *Euglena* and allies appears so similar to that of the Metazoa that it might be passed over as not distinctive; however, closer scrutiny reveals small, but consistent, differences. In the first place, the organelle is highly irregular in outline, almost as though it had become partially shriveled during preparation (Figure 9.11). While the cristae are undoubtedly flattened sacs, they are uniformly short, never extending more than halfway across the stroma, and are so broadly attached to the inner membrane that continuities between cristal and inner membranes are frequently observed. While most cristae lie with their broad surfaces parallel, a few may be set at 45 or 90° angles to the rest; consequently, face views of the sacs are commonplace in electron micrographs. In such cases, they appear irregular in outline or, more typically, bilobed.

In colorless species of euglenoids, the mitochondria are relatively more

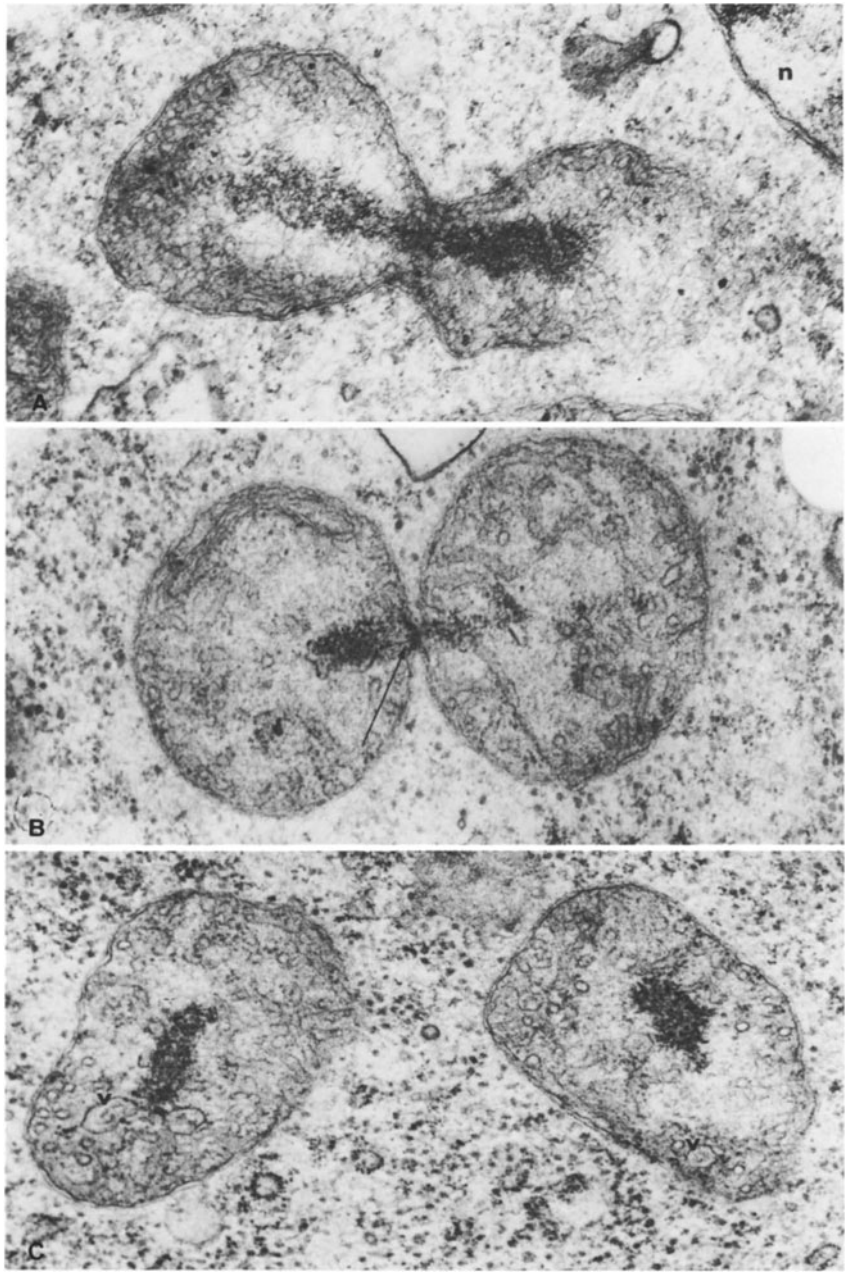


Figure 9.10. Typical mitochondria of fungi. (A) The microvillose mitochondrion of *Physarum* contains an electron-dense bar consisting in large measure of DNA, which divides as the organelle itself undergoes cleavage (B,C). All 32,000×. (Courtesy of Kuroiwa *et al.*, 1977.)

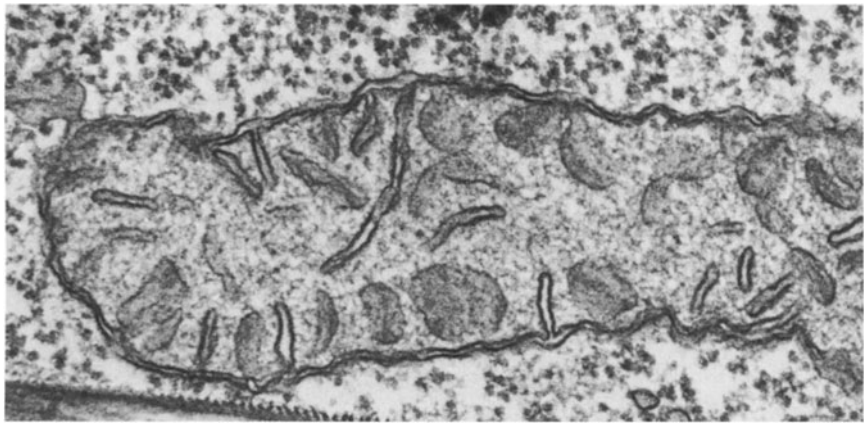


Figure 9.11. Mitochondrion of *Euglena*. This section of a mitochondrion shows the short, saclike cristae that typify this flagellate both in cross section and face view. In some species the sacs are bilobed. 72,000×. (Courtesy of Hilton H. Mollenhauer, unpublished.)

abundant within a single cell than in green forms; similarly after a green form has been treated with heat or streptomycin to inactivate the chloroplasts, the decolorized cells show a several-fold increase in the volume of mitochondria present. This increase is usually explained as reflecting the change from a phototrophic to a heterotrophic type of nutrition.

The Organelle in Other Protistans. If the organelle of *Amphidinium* may be taken as representative, the mitochondrion of dinoflagellates is relatively small, with short, rather swollen microvilli that usually are clustered close to the periphery (Dodge and Crawford, 1968). Consequently, there are broad stretches of matrix in the center of the inner compartment that are uninterrupted by cristae (Figure 9.12A). They offer a sharp contrast to the organelle of such euciliates as *Colpidium* (Foissner and Simonsberger, 1975), in which the inner compartment is nearly filled with elongate, slender microvilli (Figure 9.12B). Among radiolarians, as represented by *Aulacantha* (Ruthmann and Grell, 1964), and testaceans, including *Arcella* (Netzel, 1975), the microvilli are as long and slender as in the ciliates, but exhibit a marked tendency toward being branched. Moreover, they are much less abundant in a given mitochondrion, so that the interior is largely matrix but without the broad uninterrupted areas of the dinoflagellates. Other amoeboids, like *Gromia* (Hedley and Bertaud, 1962), have microvilli that are nearly as short and swollen as those in the dinoflagellates, but here they are slightly more elongate and much more closely set.

Among the green flagellates and filamentous algae, the mitochondrion exhibits a variety of forms. At the more primitive levels, such as *Volvulina*

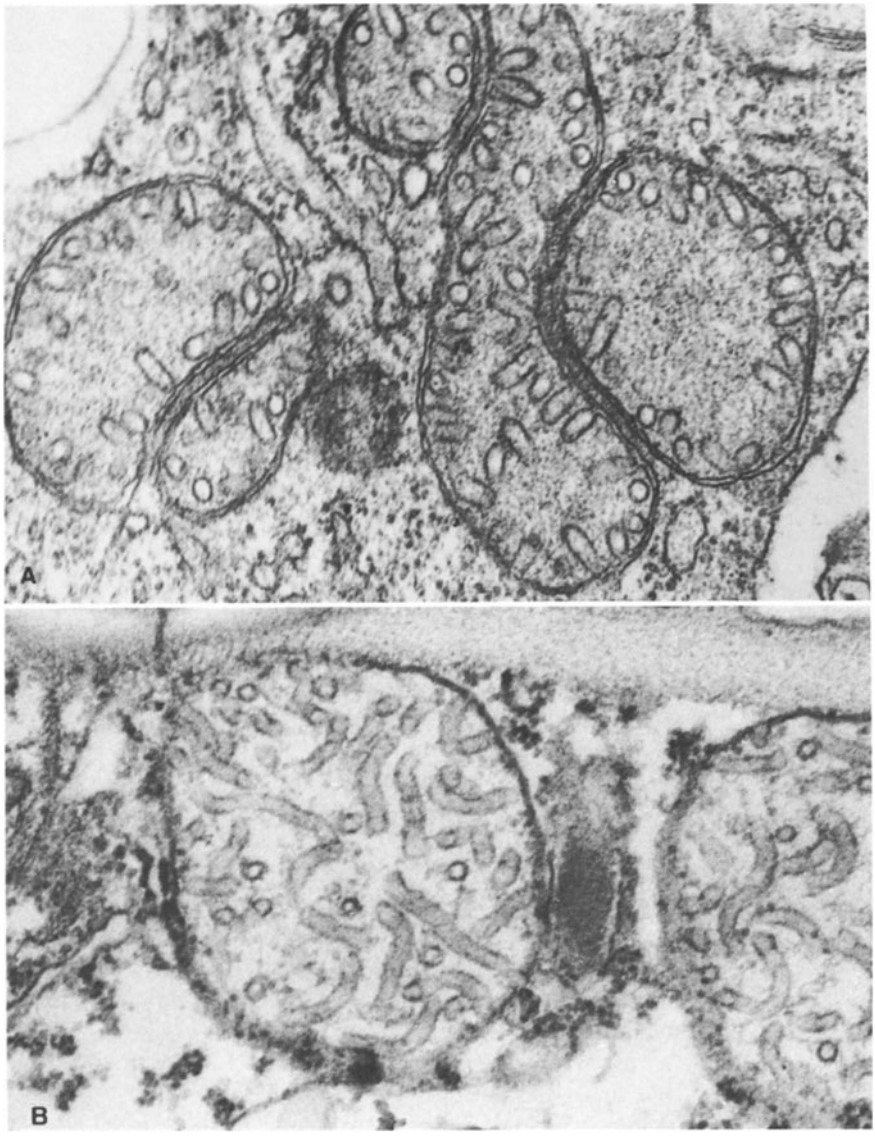


Figure 9.12. Mitochondria of various protistans. (A) The mitochondria of dinoflagellates such as that of *Amphidinium* shown here contain microvillose cristae that are exceptionally short, so that they protrude only slightly into the stroma. 50,000×. (Courtesy of Dodge and Crawford, 1968.) (B) The organelle of euciliates in general, like this one from *Colpidium*, displays the long microvilli that typify this variety in many higher eukaryotes. 56,000×. (Courtesy of Foissner and Simonsberger, 1975.)

(Lang, 1963), the microvillose type prevails, the tubules being slender and long, often reaching nearly across the inner chamber. As a rule, these are oriented directly outwards from the sides, but a few in each organelle may run longitudinally. In the less advanced of the filamentous species, the microvilli are largely replaced by paddle-shaped cristae, the blade of which in such forms as *Ulothrix* and *Klebsormidium* (Marchant and Fowke, 1977) varies in size and shape. These are often intermingled with saclike cristae and linear constituents as in metaphytans, except that these inner structures are more numerous. Quite in contrast, the mitochondria of the brown seaweeds, including *Fucus* (McCully, 1968) and *Egregia* (Bisalputra, 1966; Bisalputra and Bisalputra, 1967), approach those of certain metazoan tissues in being of the microvillose type. Usually the organelles are very densely packed with somewhat swollen, moderately long cristae. However, in *Egregia,* the tubules may be quite robust and less abundant than in *Fucus*. In the ripe sperm of these seaweeds, filamentous or tubular components extend through the entire length of the intracristal compartment (Pollock and Cassell, 1977).

The Mitochondria of Yeasts. The mitochondrion of yeasts is of particular interest, for its structure varies extensively in response to the availability of free oxygen. Under aerobic conditions it appears not too unlike that of the metazoans, with the usual double-membrance jacket and flattened cristae, but several differences may be perceived. In the first place, among the mitochondrial population of a given cell, a decided absence of uniformity is a prominent feature, whether in size, shape, number, or disposition of cristae (Thyagarajan *et al.*, 1961; Marquardt, 1962; Takagi and Nagata, 1962; Clark-Walker and Linnane, 1967; Stevens, 1974). The size, usually proportionately small for the organelle, is rarely consistent, and may at times even approach the cell in length, while the outline varies from circular to ovoid or irregular. Frequently, the cristae may be oriented both longitudinally and transversely, not to mention obliquely also, within the confines of a single organelle, and occasionally a circular arrangement can be observed (Federman and Avers, 1967). When a cristal membrane is exposed in face view, it appears as a transversely ovoid sac, often somewhat bilobed as in the euglenoids. If a number of mitochondria are examined, a discontinuity in the investing membranes is usually to be found in one or two organelles, and sometimes a set of membranes is observed to continue into the stroma as a scroll-like fold, scarcely differing from the cristae in appearance. The inconsistencies of structure and number are especially marked in the mutant forms known as petite strains (Smith *et al.*, 1969).

The presence of the discontinuities and prolongations is more readily understood after anaerobically grown yeast mitochondria are examined, especially if an obligate aerobic species is considered, such as *Candida parapsilosis* (Kellerman *et al.*, 1969). This species will not grow in the complete absence of oxygen, even in media supplemented by yeast extract, unsaturated fatty acids, and ergosterol; low oxygen tension, just sufficiently high to support growth,

thus was used to induce anaerobic effects. Under these conditions, mitochondrial sections were rare, and those few organelles present contained only a few, poorly defined cristae (Fig. 9.13A). Instead, there was an abundance of elongate, detached membranes stretched through the cell, four elongate and several short examples of which appear in the electron micrograph (Figure 9.13A). Several of these seem to be continuous with the plasmalemma, nucleus, or vacuole. Like the definitive mitochondrion, the membranes have been revealed to be centers of cytochrome *c* peroxidase activity (Avers, 1967) and, consequently, appear to have a respiratory function. When grown in the presence of chloramphenicol, mitochondria and a few cytoplasmic membranes develop in the organisms, but cristae remain few and poorly defined in the former (Figure 9.13B).

Several interpretations of the events have been advanced. One group (Linnane *et al.*, 1962; Linnane, 1965; Clark-Walker and Linnane, 1967; Watson *et al.*, 1970) presents evidence that indicates the possibility that the mitochondria were formed *de novo* by the action of a population of vesicles. These saccules, containing only an electron-transparent stroma during anaerobiosis, became filled with an electron-opaque substance following the restoration of aerobic conditions. Because mitochondria in various stages of development were later found attached to the vesicles, the latter were assumed to have produced the organelles. Other workers in the area (Marquardt, 1962; Schatz, 1963; Plattner and Schatz, 1969; Plattner *et al.*, 1970), however, show "premitochondria" in their electron micrographs; these are minute bodies structurally similar to mitochondria except they lack cristae. The premitochondria then gradually form cristal folds from the inner membrane as they increase in size to become mature organelles.

If a number of ultrastructural studies of the yeast cell are examined (including some not especially devoted to the mitochondrion so as to avoid prejudice), a somewhat different concept of the origin of the organelle in aerobic yeast unfolds (Kawakami, 1961; Thyagarajan *et al.*, 1961, 1962; Takagi and Nagata, 1962; Conti and Brock, 1965; Kellerman *et al.*, 1969; Nagata *et al.*, 1975). In these, the electron micrographs reveal a series of configurations that suggests that the open membranes characteristic of low-oxygen-tension conditions may fold over near their ends to form loose loops. As the loops elongate, open folds are then produced initially, and later as the processes continue, open vesicles result, containing few or no cristae. Finally, the vesicles mature into typical mitochondria, with more numerous cristae and completely fused membranes. A number of partly open mitochondria appear in Figure 9.13B. If this interpretation is valid, explanation is provided for the openings, gaps, or pores and other features frequently observed even in the mitochondria of aerobically grown yeast (Moor, 1964). Stepwise increases in enzymatic activities and cytochrome concentrations have been shown to accompany respiratory readaptation (Nejedly and Greksák, 1977).

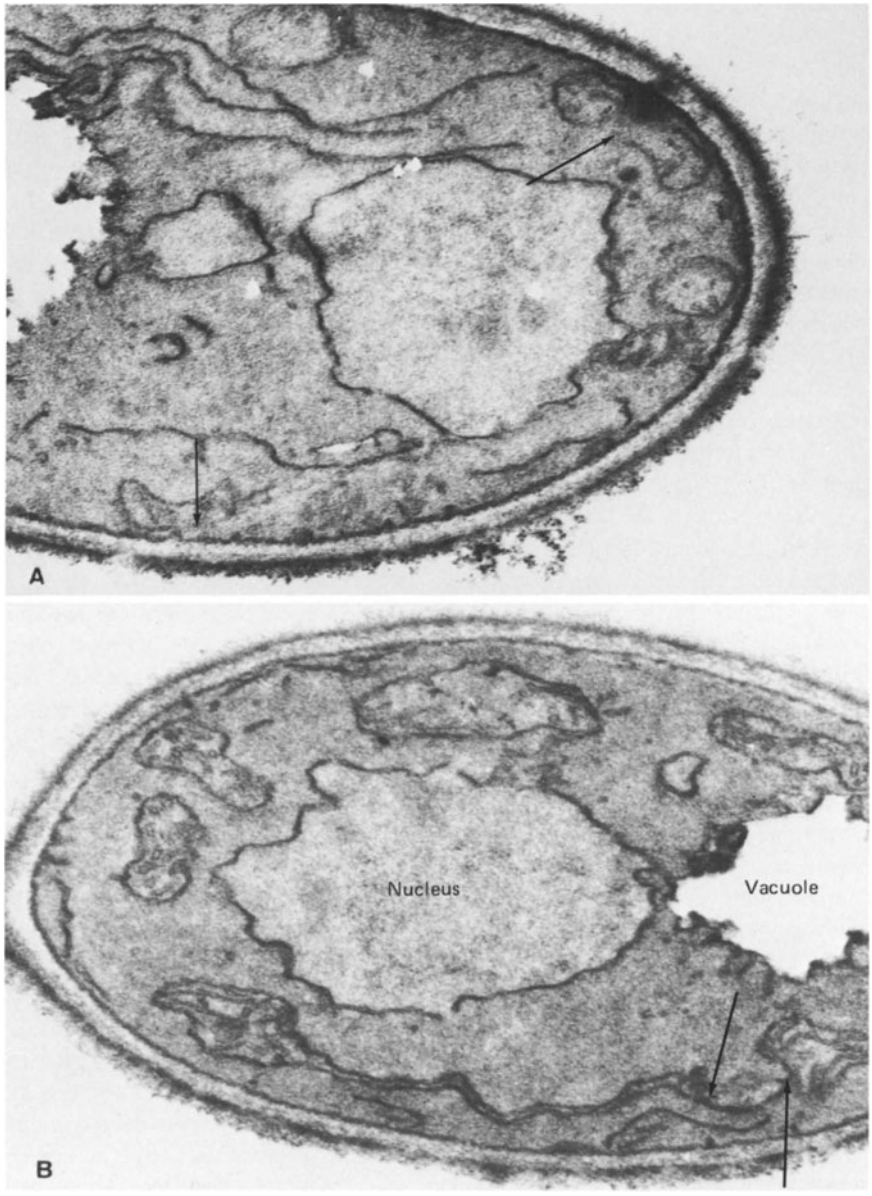


Figure 9.13. Mitochondria of yeasts. (A) The mitochondria of *Candida*, like those of other true yeasts, contain few cristae and those are often vague in outline. Moreover, many of these organelles show broad openings in the investing membranes (arrows). In this anaerobically grown specimen, mitochondria are few, whereas elongate single membranes are abundant. (B) When transferred to aerated cultures, the mitochondria increase in number while the membranes become scarce. Elongate interconnecting regions can often be noted, some of which contain openings (arrows). Both 42,500×. (Courtesy of Kellerman *et al.*, 1969.)

In electron micrographs of frozen-etched yeast cells (Moor and Mühlethaler, 1963), the surface of the outer mitochondrial membrane is seen to be irregularly covered with minute pits and a few fine furrows; its simplicity of sculpturing contrasts sharply with a comparable view in similarly prepared fungus, *Basidiobolus ranarum* (Bauer and Tanaka, 1968). Both surfaces of the outer membrane in that organism are described as showing a "fingerprint"-like structure, because of the pattern of broad ridges and furrows that constitute the membrane. It is also of interest to note here that in *Cyanidium caldarum,* a thermophilic, acidophilic alga of uncertain affinities (Rosen and Siegesmund, 1961), the mitochondria apparently develop in the fashion suggested above for yeast; it is evident that in cell shape and nuclear characteristics, this organism also closely resembles the yeasts, except that simple chloroplasts are present.

9.1.4 Behavioral and Other Properties of Mitochondria

As has undoubtedly been noted when the various figures of mitochondria were being examined, an extreme variability in size and shape characterizes this organelle. Nowhere are any two of these similar, not even where multitudes exist in the same cell. While some of the variation stems from the differing angles and areas passed through by the sectioning knife, and which therefore are more apparent distinctions than real, other contrasts in configuration or extent are actual. The basis for the variability becomes more comprehensible as behavioral and certain organizational traits are discussed.

The Motility of Mitochondria. Many organelles have a more or less fixed location in the cell, such as dictyosomes adjacent to the flagellar centriole, and chloroplasts laterally, but not so the mitochondrion. Cinematomicrography and other techniques have revealed it to be a most actively motile structure, as first shown many years ago by Lewis and Lewis (1914). In *Euglena* continuous activity was observed in the chondriome as the various parts elongated, branched, fragmented, and fused (Leedale and Buetow, 1970). As acid phosphatase had been demonstrated to be present in the organelles, it was suggested that this enzyme might be involved in the fragmentation and fusion processes, for the mitochondria of cells starved for more than 7 days lacked both these abilities and the enzymes.

The mitochondria of *Xenopus laevis* tadpole heart epithelial cells have been studied by cinematomicrography (Bereiter-Hahn and Morawe, 1972) and found similarly to move saltatorially. Although cytosol movement was enhanced by HCN, the movements of these organelles ceased, nor were they increased when ATP was added as were those of the cytoplasm. Consequently, their motility is independent of that of the rest of the cell. Mitochondria in cells influenced by 2,4-dinitrophenol displayed three different maxima in their velocity distribution curves, suggesting the existence of heterogeneity among these organelles, a topic discussed more fully in a later section. Through use of

phase-contrast microscopy with the same type of cells, mitochondria were noted to travel at speeds up to 100 μm/min, their movements falling into four categories (Bereiter-Hahn, 1978): (1) alternating extension and contraction; (2) formation of lateral branches; (3) peristaltic wavelike action along the length of the organelle; and (4) contraction and expansion of individual regions.

The Mitochondrion as a System. Some of the apparent variation in size and appearance also may be attributable to a condition that has thus far been described in only a few organisms, but which eventually may prove to be a nearly universal characteristic of cells in general, except perhaps those of multicellular plants and animals. Like the tubular interdictyosomal connections recently found to unite the Golgi bodies of a cell into a single system, mitochondria also have proven to be interconnected. However, in the present case, the connections are actually continuities between expanded and contracted portions of the same body. Three-dimensional models derived from 80 to 150 consecutive serial sections of entire yeast cells showed that all the mitochondrial profiles were sections through a single, highly branched, very irregular tubular structure that traversed the entire cell just beneath the plasmalemma and continued into the forming bud (Figure 9.14; Hoffman and Avers, 1973).

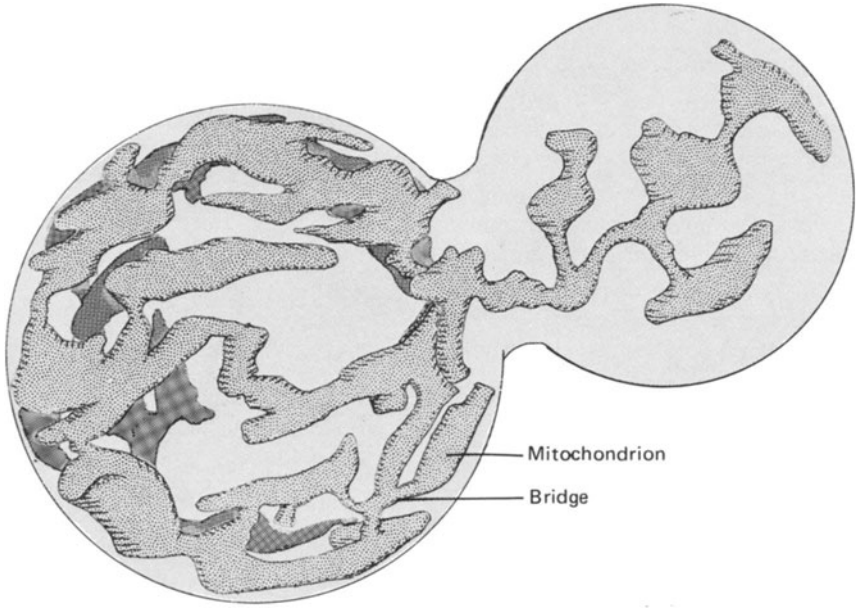


Figure 9.14. The mitochondrial system of yeast. The mitochondria of yeast are joined into a single continuous system. (Based on Hoffmann and Avers, 1973.)

Apparently cristae extend throughout the length of the mitochondrion; thus small mitochondria are merely sections through a neck that interconnects two expanded regions. Because in life the entire body as a unit and its separate parts individually expand and contract, elongate and shorten, bend and straighten, no two cells can ever be similar in regards to the size, shape, or number of this organelle.

The condition just described for the typical yeast cell may not be repeated in precise fashion throughout the biological world, but the general pattern probably is. In *Chlamydomonas,* reconstruction of a cell model from numerous sequential serial sections demonstrated the existence of eight mitochondrial bodies (Arnold *et al.*, 1972; Schötz *et al.*, 1972). As in the yeast, these were found to be contorted, branched, elongated tubular structures, most of which were concentrated below the plasmalemma, while the rest seemed to be largely associated with the chloroplast. Although the mitochondrial system, or chondriome, of *Euglena* has been studied only in living cells (Leedale and Buetow, 1970), that investigation demonstrated similar continuities between the respiratory organelles. Often the interconnections, and even the mitochondria themselves, were threadlike—the "threads" and "mitochondria" are actually the same thing, the diameters and form being temporary states common to the entire system.

9.1.5. Highly Modified Mitochondria

In addition to the various morphological modifications in the crista that have been presented, the mitochondrion as an entity has undergone specializations in structure. Moreover, at least in one general type, the organelle has given rise to a distinct part that is so highly modified that it lacks all the characteristics of its parent body.

The Mitochondrial Helix of Spermatozoa. In the midpiece of the metazoan spermatozoon, that is, the region just posterior to the head, is a series of greatly elongated mitochondria, usually twisted into a compact helix around the flagellar basal region. Among mammals a variable number of rows of mitochondria commonly comprise the helix, and the organelles themselves may show slight internal modifications as well (Threadgold, 1976). In bull sperm, three or four rows are present that together form 64 gyres around the flagellum, whereas in rabbit four or five rows encircle the flagellum 41 times (Figure 9.15; Phillips, 1977). Contrastingly, rhesus monkey spermatozoa have only a double helix, as do mouse sperm. In some instances, fusion may take place between adjacent mitochondria, but such is not the case in rat sperm (André, 1962).

Among invertebrates, mitochondria occasionally remain within the sperm head at the posterior of the nucleus rather than spiraling along the flagellum, but several striking variations on this theme have been described. In a pulmonate snail, *Testacella haliotidea,* the mitochondria are very numerous during


A
B
C

Figure 9.15. Modified mitochondria of mammalian sperm. At the midpiece of mammalian spermatozoa, mitochondria are arranged in helices around the flagellar sheath, as shown in these electron micrographs of platinum-carbon replicas. Shown are the midpieces of bull sperm (A, 15,000×), mouse (B, 10,000×), and rhesus (C, 19,000×). (D) At higher magnification, the mitochondria of Chinese hamster are seen to be precisely shaped and arranged. 41,000×. (All courtesy of Phillips, 1977.)

spermatogenesis; late in the spermatid stage, still within the head, they fuse into a single mass, out of which later is produced one gigantic derivative. Still later this itself undergoes metamorphosis, the final product being a paracrystalline rod that surrounds the flagellar base; most of the substance of the rod is protein, arranged in numerous parallel tubules about 90 Å in diameter (André, 1963). A second example occurs among the insects, in which taxon the mitochondria of the sperm of a sphinx moth (*Macroglossum stellatarum*) undergo a comparable metamorphosis. However, the end product is quite different, for it consists of a thick-walled, elongate rod that encloses a structured electron-transparent matrix surrounding the flagellum.

The Kinetoplast. Among the trypanosomatids and their relatives, the bodonids, a peculiar structure is present near the flagellar centriole, one of several organelles formerly referred to as the blepharoplast by light microscopists. Now it has been revealed by electron microscopy usually to be a rodlike mass (Figure 9.16), circular or ovate in cross section, consisting of densely packed transverse fibers (Pitelka, 1961; Rudzinska *et al.*, 1964). Also it has been shown to be part of a single mitochondrion and to consist largely of DNA, the combination of organelle and rod now being known as the kinetoplast (Trager, 1964; Simpson, 1972). The Feulgen-positive rod of DNA has proven to be fairly consistent in width for a given species; in *Crithidia fasciculata,* for example, its diameter is between 0.23 and 0.29 μm (Anderson and Hill, 1969).

The kinetoplast contains the largest known deposit of DNA outside of the

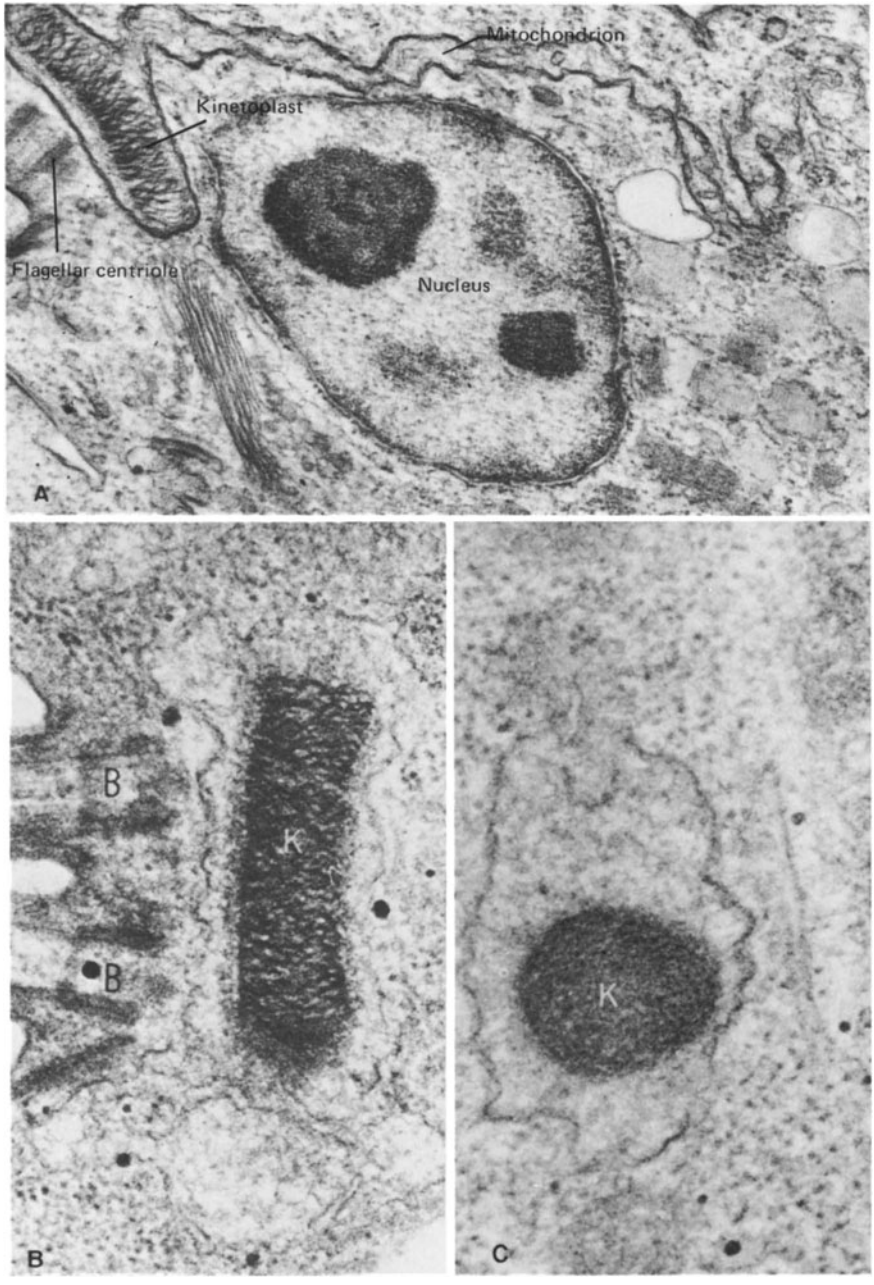


Figure 9.16. The kinetoplast, a derivative of the mitochondrion. (A) The kinetoplast of this *Trypanosoma conochina* cell is seen to be an expanded portion of the mitochondrion enclosing an electron-opaque body. 35,000×. (Courtesy of Milder and Deane, 1967.) Longitudinal (B) and transverse (C) sections of the kinetoplast (K) of *Crithidia* reveal similar structure and orientation close to the flagellar centrioles (B). Both 40,000×. (B and C, courtesy of Hill and Anderson, 1969.)

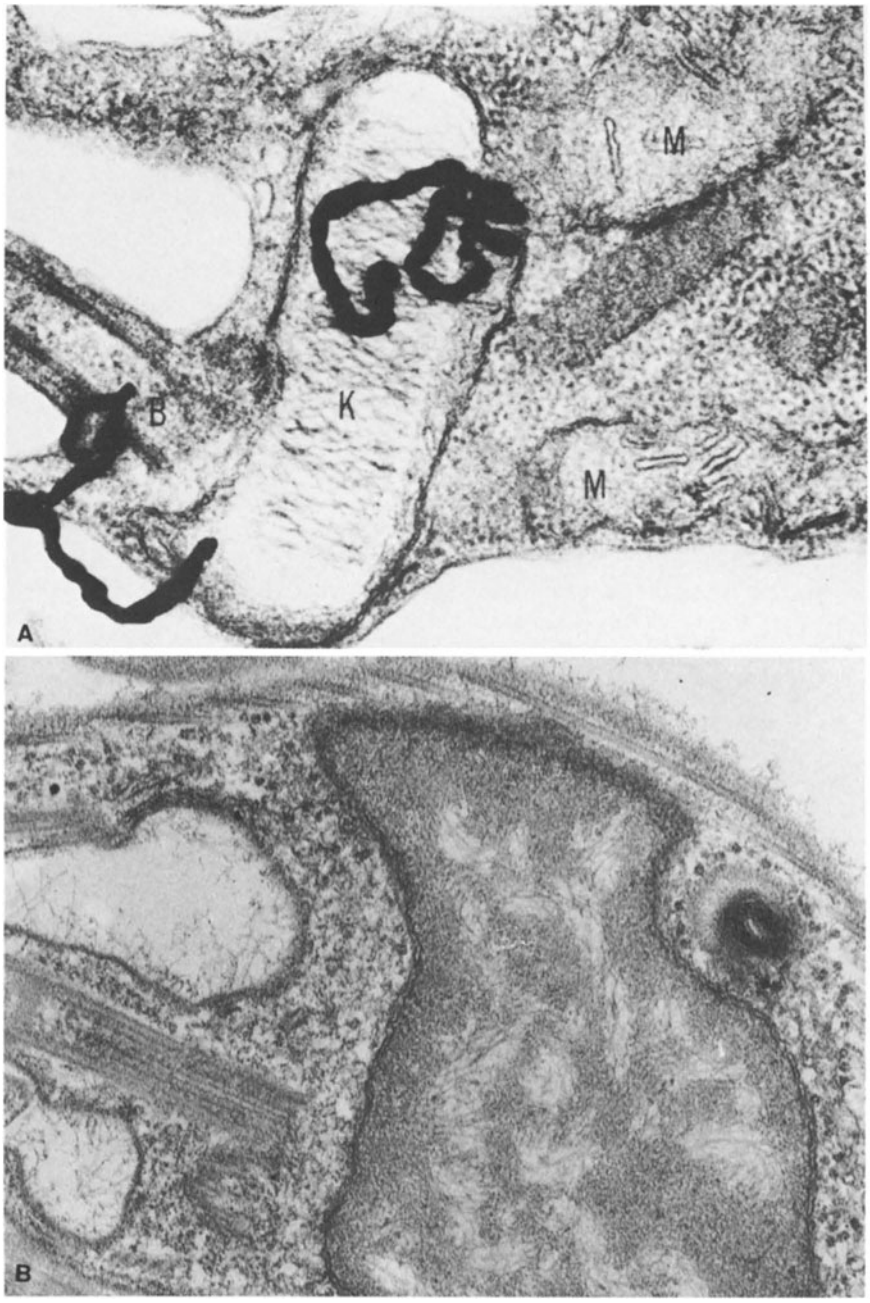


Figure 9.17. DNA of the kinetoplast. (A) This specimen of *Crithidia* has been pulsed with labeled thymidine, the activity being confined to the kinetoplast (K). 80,000×. (Courtesy of Hill and Anderson, 1969.) (B) In *Cryptobia* the DNA is distributed widely throughout the kinetoplast, but especially near the flagellar base. 70,000×. (Courtesy of Vickerman, 1977.)

nucleus (Figure 9.17), but its role in the cell has not been deciphered as yet. Nor has it been determined why it is so closely associated with the flagellar base (Vickerman, 1977). That it is not entirely essential is clearly indicated by its normal absence in *Trypanosoma equinuum* and in some strains of *T. equiperdum* and *T. evansi* (Simpson, 1972). Although part of its DNA is in the form typical of many other mitochondria, as described later, the bulk of the molecules are atypical to such an extent that the ability to serve a genetic function has been questioned (Borst and Fairlamb, 1976).

The kinetoplast of the bodonids differs somewhat in form from the corresponding organelle of the trypanosomatids. In *Bodo* perhaps the greatest distinction is that the kinetoplast is continuous with the so-called "cord" of mitochondria that encircles the cell, and not merely a rod surrounded by a mitochondrial remnant (Hill and Anderson, 1969). Instead of the short cristae found in *Trypanosoma,* opaque granules fill the space between the DNA fibrils and the limiting membrane (Pitelka, 1961). Another member of the group has quite a different arrangement but varies between the dimorphic forms that occur in this species, the common "thin" and the rare "broad" forms. The latter have the kinetoplast DNA concentrated in a mass near the flagellar centriole (Vickerman, 1977), but in the more frequent arrangement the DNA is dispersed throughout the mitochondrial network.

9.2 MOLECULAR ORGANIZATION OF MITOCHONDRIA

As already brought out, the foremost enzymes of the metazoan mitochondrion are those involved in oxidative phosphorylation, including the electron-transport chain and many enzymes of the citric acid cycle, but excluding those of glycolysis. Hence, the mitochondrion stands out as the ATP-producing center *par excellence* of the cell.

9.2.1. Enzymes of the Mitochondrion

Energy-Requiring Activities. Within this organelle a number of reactions that utilize ATP also are known to occur, including the synthesis of protein discussed in a later section. After their biosynthesis, certain proteins are phosphorylated by a protein kinase located on the stroma side of the mitochondrial inner membrane of mammals (Vardanis, 1977), as well as those of yeast (Rogobello *et al.*, 1978). Among other synthetic activities may be listed the formation of phosphatides, hippuric and *p*-aminohippuric acids (Kielly and Schneider, 1950; Leuthardt and Nielson, 1951), and citrulline (Siekevitz and Potter, 1953). Carboxylations and phosphorylations of nucleoside diphosphates in general are likewise localized here (Herbert and Potter, 1956). In addition, amino acid metabolism may be a particular function of the mitochondrion,

because transaminases (Hird and Rowsell, 1950), glutaminase I (Shepherd and Kalnitsky, 1951), and glutamic dehydrogenase are present in abundance (Hogeboom and Schneider, 1953; Schneider, 1959). Among specific amino acids actually known to be oxidized are thyroxine and triiodothyronine.

The metabolism of glutamine in avian liver provides an interesting insight into some mitochondrial processes that are not energy related. The synthetase of this amino acid was found to be located within the matrix of this organelle (Vorhaben and Campbell, 1977), together with dehydrogenases of malic and glutamic acids and that of NADP-dependent isocitric acid. Glutamine synthetase parallels carbamyl phosphate synthetase I functionally but occurs in the liver of uric-acid-secreting (uricotelic) species instead of urea-secreting (ureotelic) ones as the latter does (Vorhaben and Campbell, 1972; Campbell, 1973; Campbell and Vorhaben, 1976). Both enzymes mediate the initial steps in the detoxification of ammonia through the synthesis of either uric acid or urea. When glutamic acid is catabolized by glutamic acid dehydrogenase, the resulting ammonia is used in the synthesis of citrulline by way of carbamyl phosphate or, in uricotelic forms, it is employed in the production of glutamine through the action of glutamine synthetase. In both cases the products then leave the mitochondria for final conversion into the excretory compounds. In contrast to the glutamine synthetase, which is localized in the matrix, phosphate-dependent glutaminase is confined to the mitochondrial outer membrane in avian liver; in rat liver, however, it is found in the interior of the organelle (Curthoys and Weiss, 1974). The enzyme paralleling glutamine synthetase functionally in rat liver, carbamyl phosphate synthetase, has been reported to be contained completely within the inner membrane, where it makes up about 15% of the total mitochondrial protein (Clarke, 1976a). Its molecular weight has proven to be in the neighborhood of 165,000.

Schneider (1959) lists also a number of oxidases that are localized in mitochondria. Among the more important are those of mesotartrate and sarcosine, cholestrol and other steroids, all the fatty acids, xylitol and other polyols, itaconate, and kynureinine. The inorganic pyrophosphate produced by the β oxidation of fats or activation of amino acids is known to be hydrolyzed by a pyrophosphatase located on the inside surface of the inner membrane (Schick and Butler, 1969).

Heterogeneity of Enzymes. In multicellular organisms, tissue differences in mitochondrial enzymes occur, just as has been the case of the other organelles that have already received attention. One investigation that compared several dehydrogenase activities in mitochondria of four rat tissues (Ohkawa *et al.*, 1969) showed that the liver and kidney organelles were nearly equal in being more highly reactive for β-hydroxybutyric and glutamic acid dehydrogenases than either white or brown adipose tissue, and that kidney ranked highest in succinic acid dehydrogenase. However, mitochondria of brown adipose tissue were at least ten times as active in α-glycerophosphate

dehydrogenase as the others, white adipose tissue being the least reactive for the enzyme. This latter difference is all the more remarkable, because the substrate is known to be a precursor for glycerophosphatides and triglycerides, substances important in lipid metabolism. In contrast, the enzymes for another set of reactions associated with fat metabolism, the production of ketone bodies, were found to be located in liver cell mitochondria (Chapman *et al.*, 1973).

Even in such unicellular organisms as bakers' yeast, the mitochondria are not all identical, in spite of their being united into a single system. Schatz (1963) was the first to distinguish two distinct populations of these organelles in *Saccharomyces*. One of these carried a high degree of succinate-cytochrome *c* oxidoreductase activity, whereas the second group was highly active in succinate dehydrogenase. This presence of at least two types was later confirmed by means of biochemical and quantitative electron microscopic procedures (Matile and Bahr, 1968); in addition, the two types were reported to differ noticeably in relative weight. HeLa cell mitochondria also have been demonstrated to be heterogeneous in function (Storrie and Attardi, 1973).

9.2.2. Mitochondrial Membrane Activities

Mitochondrial Membrane Structure. As the numerous concepts regarding the structure of membranes, including those of the mitochondrion, have already been discussed (Chapter 1), here only the one that appears to apply particularly well to mitochondrial properties receives attention, that of Sjöstrand and his co-workers (Sjöstrand, 1977, 1978; Sjöstrand and Cassell, 1978). In brief, the most pertinent characteristics are (1) the outer, inner, and cristal membranes are each distinctively structured (Figure 9.18). Hence, the cristae are not to be viewed as mere outpocketings of the inner membrane. (2) The membranes are largely (75%) protein. (3) The inner membrane has lipid on only one surface, that bordering the matrix. (4) The cristal membranes are 150 Å thick, but so closely appressed that a single crista is 300 Å in thickness. (5) The outer membrane readily permits the passage of water, whereas the inner one limits water penetration, possibly through lipids sealing the interstices between protein molecules. (6) In these proteinaceous membranes, both polar and nonpolar regions alike can be exposed to the surface or just as readily embedded within the interior, wherever their specific properties may be needed. (7) No outer compartment nor intracristal spaces are present. This view of the enclosing membranes being closely applied to one another affords an explanation of the earlier observation that freeze-fracture preparations do not disclose the outer face of the inner membranes (Ruska and Ruska, 1969).

Substructure of the Membranes. Using negative staining techniques on isolated beef heart mitochondria, Fernández-Morán (1961) found a macromolecular repeating unit in both the cristae and the inner membrane. Later, these units, called subunits or elementary particles, were shown by Fernández-

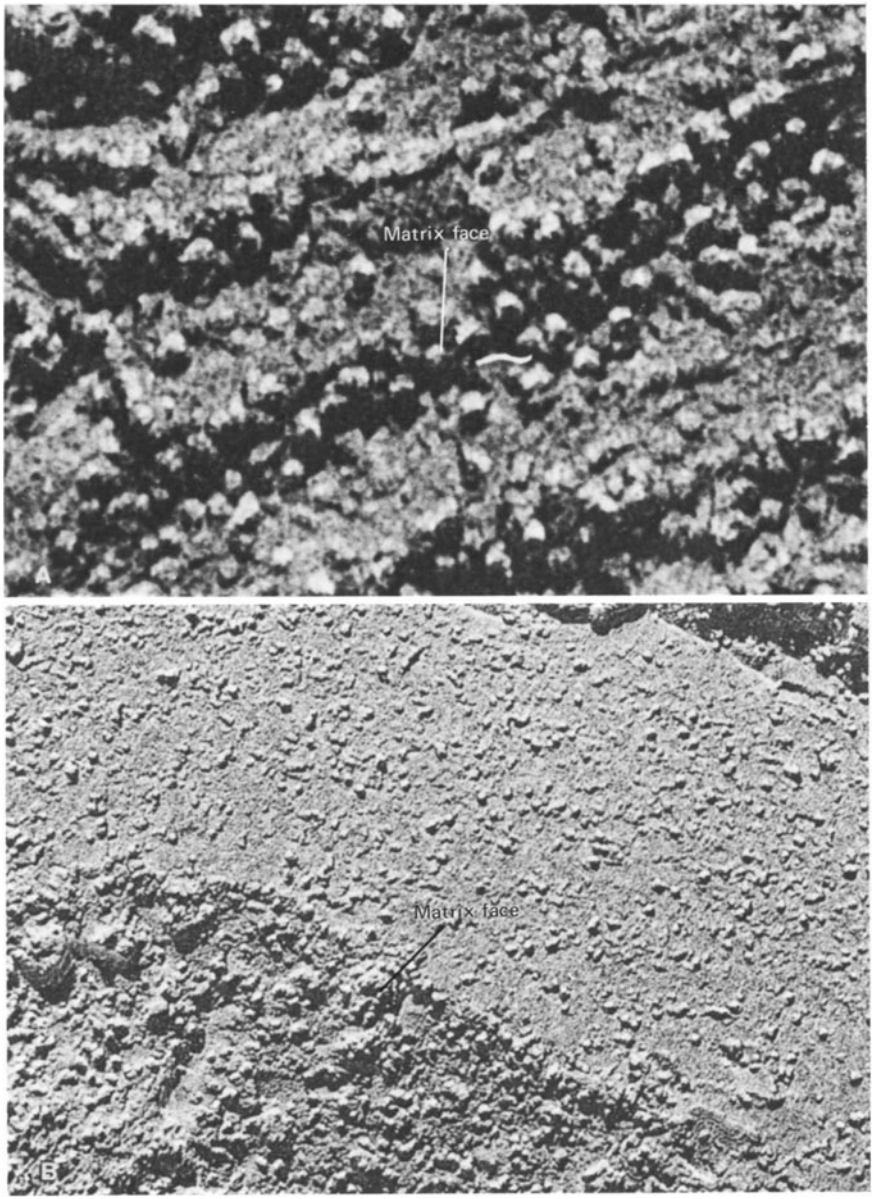


Figure 9.18. Mitochondrial membrane substructure. (A) Freeze-fracture preparation cut obliquely through the cristae; the matrix surface is densely covered with coarse particles. 380,000×. (B) Same but exposing the matrix surface of the inner membrane of the envelope; large convexities and irregular particles are present. 130,000×. (Both courtesy of Sjöstrand and Cassell, 1978.)

Morán *et al.* (1964) to consist of three parts: a more or less spherical or polyhedral head, 80 to 100 Å in diameter, a slender stalk, 30 to 40 Å wide and approximately 50 Å in length, and a base piece of 40 × 100 Å dimensions. The base pieces formed an integral part of the outer electron-dense lamella of the membrane, while the heads projected into the stroma in regular arrays. From the same source, subunits resembling these elementary particles in size later were isolated and found to contain complete electron-transport chains. Because cytochromes *a* and a_3 require dissolved O_2 in their reactions, these researchers believed the end of the chain to be localized in the head piece projecting into the stroma. On the other hand, because the dehydrogenases, some of the nonheme iron proteins, coenzyme Q, and cytochrome *b* interact with NADPH or succinic acid, they were considered to be located in the base piece and the remainder of the transport chain in the stalk.

Later Green and Perdue (1966) removed the head pieces of the subunits by sonication; then, by ultracentrifugation, they separated fractions containing inner and outer mitochondrial membranes, respectively. Upon testing, the entire electron-transport system proved to be localized in the base pieces of the cristae and inner membrane. The head pieces, these biochemists suggested, were not all alike but actually were different kinds of enzymes, including ATPase as demonstrated by Racker and Conover (1963). Later it was reported that the stalks consisted of fibrous proteins (Hall *et al.*, 1969).

On the other hand, in the mitochondrial outer membrane the subunits lacked both stalked head pieces and electron-transport activity (Green and Perdue, 1966; Parsons *et al.*, 1966). In this membrane the particles were about 90 Å in diameter and seemed to consist of various enzymes of the citric acid cycle, as well as those of fatty acid oxidation and elongation, and of hydroxybutyrate and amino acid oxidation (Allmann *et al.*, 1966).

The ATPase–ATP synthase complex of enzymes has been demonstrated to be asymmetrically oriented in the inner* mitochondrial membrane (Hootman and Philpott, 1979), the factor F_1 being located in the matrix side (Racker, 1970). In the processes of ATP metabolism, thiol groups of the several enzymes play important roles (Senior, 1973). Those involved in the mechanism of ATP synthesis have now also been localized in a nonpolar area on the matrix surface of the inner membrane (Blanchy *et al.*, 1978). It is of interest to note that the gene for the ATPase in yeast has now been sequenced (Macino and Tzagoloff, 1979).

In addition, similar studies have revealed an orientation of various components in the same membrane but in the opposite direction (Krebs *et al.*, 1979). For instance, a total of seven classes of polypeptides were separated by dodecyl sulfate gel electrophoresis (Clarke, 1976b), having molecular weights of

*It should be borne in mind that the term inner membrane in these reports includes the cristal membrane as well as the inner investing one.

130,000, 87,000, 73,000, 31,000, 26,000, 16,000, and 10,500, respectively. Although all of these were localized on the exterior surface of the inner membrane, their specific functions were not ascertained, but they were assumed to be involved in transmembrane transport. However, complex II of mitochondrial membrane, a fragment containing succinic acid-ubiquinone reductase activity and two polypeptides, has been reported as spanning the inner membrane (Merli *et al.*, 1979). The two polypeptides, called CII3 and CII4, had molecular weights of 13,500 and 7000, respectively; the first of these was found to be located on the outer surface of that membrane, while the succinic acid dehydrogenase flavoprotein was on the matrix side.

The sites of some enzyme activities have now been localized in submitochondrial fractions of plant tissues. Intact mitochondria from the endosperm of castor bean were isolated, ruptured, and separated into inner and outer membrane fractions, each of which was then examined for the presence of certain enzymes (Sparace and Moore, 1979). The results indicated that the synthesis of phosphatidylglycerol, CDP-diglyceride, and phosphatidylcholine occurred in the inner membrane, whereas that of phosphatidic acid took place in this as well as the outer membrane. Furthermore, the phospholipids themselves have been shown to be asymmetrically distributed within the inner membrane itself (Krebs *et al.*, 1979).

To refer back to a matter discussed in the first chapter, it is evident that the two membranes investing this single organelle present further evidence negating the concept of universality of structure among membranes. The protein content in the form of enzymes and coenzymes of one bears no resemblance to that of the other, any more than does the form of the subunits that compose each one. Furthermore, both membranes, like those of the chloroplast but unlike most others, appear in freeze-etch preparations to lack a central lipid layer (Staehelin, 1968).

Miscellaneous Membrane Enzymes. In addition to the enzymes enumerated above, the mitochondrial membranes contain a number of others that have been identified but not localized in further detail. One of the more important is an actinomyosinlike protein (Ohnishi and Ohnishi, 1962) that probably is responsible for much of the organelle's marked abilities of elongation and contraction. Moreover, a structural protein with a monomeric molecular weight of around 22,000 has been isolated (Criddle *et al.*, 1962); immunological tests show it to be similar in the mitochondria of such diverse organisms as yeast, *Neurospora,* and beef. *In situ,* the protein is polymeric and insoluble in water, but treatment with alkali or anionic detergents converts it to the soluble monomer. Upon restoration of the pH to neutrality or removal of the detergent, polymerization proceeds rapidly, during which processes the protein can combine with phospholipids to form a complex (Fleischer and Klouwen, 1960). With any purified cytochrome, whether *a, b,* or *c,* the structural protein similarly can unite to form water-insoluble complexes in a 1:1 molar ratio. Com-

bination of the structural protein and cytochrome *b* is a slow process, requiring a minimum of 4 hr. When thus combined, cytochrome *b* undergoes a significant alteration in its oxidation–reduction potential (Goldberger *et al.*, 1962), changing from $E_0' = 0.34$ V to one more positive than 0.0 V.

Long-chain fatty acids from dietary sources play a role of considerable importance in the composition of the mitochondrial membranes and even the functioning of the organelle (Awasthi *et al.*, 1971; Blomstrand and Svensson, 1974). To cite one instance, the erucic acid of rapeseed has been reported to be incorporated preferentially into cardiolipin, an integral constituent of cytochrome *c* oxidase, with resulting decrease in mitochondrial ATP synthesis (Houtsmuller *et al.*, 1970; Clandinin, 1976).

Transport across the Mitochondrial Membranes. Mitochondria possess marked abilities in accumulating cations, including monovalent ones like K^+ and divalent ones such as Ca^{2+}, Mn^{2+}, and Sr^{2+}, as well as anions, among them phosphate, arsenate, acetate, sulfate, and those of the citric acid cycle. These capabilities, together with the energy pathways present, make the mitochondrion an ideal system for studying movement across living membranes. Such studies have been rather numerous, especially since a number of agents whose effects upon specific sites along the pathways have become known.

While such investigations are not pertinent to the present account, it is not surprising that they have afforded some insight into mitochondrial function. For example, Siekevitz and Potter (1953) observed an increased rate of respiration in mitochondrial suspensions upon addition of small amounts of Ca^{2+} in the absence of ADP. In later experiments, Chance (1964, 1965) found that this elevation of the respiratory rate did not endure indefinitely but returned to the resting rate after a period of time. In a complete stoichiometric study of these Ca^{2+}-induced "respiratory jumps," Rossi and Lehninger (1963) showed that between 1.7 and 2 Ca^{2+} cations were required to activate each respiratory site and that virtually all the calcium was accumulated by the mitochondrion during the period of the respiratory jump. Lehninger (1966) demonstrated that this calcium was not irreversibly retained within the organelle but was maintained at a steady state by equal rates of Ca^{2+} ingress and egress through the membranes. Although the mitochondria most thoroughly studied were derived from rat liver, those of other cell types showed similar Ca^{2+} relations; sources included kidney, brain, heart, and skeletal muscle, maize and bean seedlings, the fungus *Neurospora crassa,* and yeasts. Chappell *et al.* (1963) and Carafoli (1979) reported comparable reactions for Sr^{2+}, and the former investigators for Mn^{2+} as well, but Mg^{2+} did not induce respiratory jumps nor was it accumulated within the mitochondrion.

Addition of larger quantities of Ca^{2+} to the medium induced massive accumulation of the cation by respiring organelles, a process blocked by 2,4-dinitrophenol or cyanide, but not by the inhibitor oligomycin (DeLuca and Eng-

strom, 1961; Vasington and Murphy, 1962; Brierley and Slautterback, 1964). Rossi and Lehninger (1963) established that Ca^{2+} accumulation was coupled to inorganic phosphate uptake in a ratio of 1.67 Ca^{2+}: 1.0 P_i. With these higher concentrations of Ca^{2+}, mitochondria did not phosphorylate ADP; consequently, massive Ca^{2+} loading may be viewed as a process alternative to oxidative phosphorylation of ADP—explaining why calcium ions have traditionally been considered an uncoupling agent. Ultrastructural investigations of treated mitochondria show the calcium phosphate to be deposited within the stroma; hence, it is clear that chemicals can be transported directly across both investing membranes. Strontium together with organic phosphate may be accumulated in the same manner as calcium, but massive loading of either substance leads to mitochondrial damage.

Molecules as large and complex as amino acids and proteins, including cytochrome *c,* are also apparently conducted into the mitochondrion from outside sources, as becomes apparent in later discussions, but the mechanisms involved to date seem to have been explored to only a limited extent (Chua and Schmidt, 1979; Schatz, 1979). Similarly, such large molescules as NADH · H are known to be employed in various reactions, including gluconeogenesis, within the cytosol, although the chemical named has been established as being synthesized solely in the mitochondrion (Krebs *et al.*, 1967). As mitochondria show low permeability for both NAD and NADH · H, some carrier mechanism may be present. The investigators cited suggest that the carrier, possibly the malate-oxaloacetate system, may transfer reducing equivalents of intramitochondrially generated NADH · H rather than the substance itself. If so, one concept or another seems destined for change—either that of the direct involvement of NADH · H in the cytoplasmic reactions or of its formation occurring solely in the mitochondria.

The transfer of the acetyl group of acetyl-CoA from the inner compartment through the inner membrane also is an essential step in the *de novo* synthesis of fatty acids from glycolytic precursors. Investigations into the mechanism for transport of such radicals have shown that most of the activity is by way of citric acid (Watson and Lowenstein, 1970; Lowenstein, 1971). However, more recently it has also been established that between 15 and 20% is transferred through the inner membrane by an additional pathway, probably involving free acetate (Walter and Söling, 1976).

Still another mechanism for transport through the mitochondrial membranes has been uncovered, one that pertains to proteins made in the cytoplasm and then imported into the mitochondrion. The proteins involved in this investigation were subunits of the F_1 ATPase of the yeast organelle, the three largest of which were found to be synthesized as still larger precursors (Maccecchini *et al.*, 1979). These then penetrated both enclosing membranes, apparently undergoing trimming as they did so, for only the mature subunits occurred within the inner compartment.

9.2.3. Other Molecular Aspects

Mitochondrial Aging. A number of investigations have been directed at the problem of aging in mitochondria (Waite *et al.*, 1969; Parce *et al.*, 1978), the results of many of which suggested that endogenous phospholipase A_2 might be involved in the observed deterioration of energy-linked functions in the organelle. The first report cited showed that a direct relationship existed between the activity of this enzyme and irreversible swelling of the mitochondrion, which change has been long associated with loss of energy-related processes. Moreover, Scarpa and Lindsay (1972) obtained evidence intimating that the enzyme was likewise responsible to some degree for the decay of respiratory control that accompanies aging. In the aging processes, two main stages have been recognized (Parce *et al.*, 1978), the first involving a reversible loss of energy-linked functions, including a decline in the level of ATP, a decreased ability to reacylate monoacylphospholipids, and a decline of fatty acid oxidation rates. Both of the last two effects are really derived from the diminution of the ATP content. The second phase is marked by the irreversible loss of energy-linked activities, including the ability of the inner membrane to produce the energized configuration. Apparently, this final phase is in part the consequence of phospholipase A_2 activity, which results in the loss of phospholipids from the membrane.

Composition of the Stroma. Because investigations into the macromolecular composition of mitochondria have centered attention upon the membranes, the matrix remains relatively poorly explored. It should not be supposed, however, that all the enzymes enumerated in the preceding section are thus implied to have been established as being part of the membranes—only those of ion and metabolite transport and the majority of those involved in protein synthesis and in respiration have been clearly localized as being there. But few of the remainder have actually been consigned to the matrix. Among them are the two motile constituents of the electron-transport chain, that is, coenzyme Q and cytochrome *c*. This pair, unlike the other members, has been suggested by Green and Wharton (1963) as not being fixed so that they migrate among the rest and react by random collision. Another set believed to be in the matrix, or perhaps only weakly attached to the membranes, are the dehydrogenases of the citric acid cycle, because they are generally lost when mitochondria are fragmented. The matrix also contains an extensive pool of free amino acids (Roodyn *et al.*, 1961; Truman and Korner, 1962; Roodyn, 1965), and amino-acid-activating enzymes, many, if not all, of which differ sharply from their counterparts in other portions of the cytoplasm (Barnett *et al.*, 1967). Although evidently different in molecular structure, the system can serve to replace cytosol in ribosomal systems of protein synthesis (Truman and Korner, 1962).

Ultrastructural studies have clearly established that the matrix includes another component, one whose presence in the organelle holds several impor-

tant lessons for biologists and biochemists alike. During the past several decades, it had been the practice of investigators in general to insist that all the DNA of the cell was contained in the nucleus. When on occasion DNA was well authenticated as being in the cytoplasm (for references see Gahan and Chayen, 1965), the results were generally passed off as being in atypical cells. As Roodyn (1967, p. 145) has pointed out, the history of the discovery of this substance within the mitochondrion "is an interesting example of how an oversimplified view can easily develop into a false dogma that can then retard the development of further research." Roodyn continues by stating "that of the thousands of studies on mitochondria in the fifties and early sixties, there were extremely few reports of DNA assays in the mitochondrial fraction." The remaining studies merely assumed that the 90 to 95% recovered in the nuclear fraction represented the total recoverable by the procedures. Unfortunately for the progress of science, false dogmas and oversimplifications are more readily perceived in retrospect than through the eyes of the present. They can only be ferreted out by continual reexamination of concepts.

The first to break with tradition were Chèvremont and his co-workers (1959) who, by employing autoradiographic and cytochemical techniques upon fibroblasts in tissue culture, demonstrated the presence of DNA in the mitochondrion. However, all doubt on the subject was removed only by the electron microscopic studies by Nass and Nass (1962), whose micrographs clearly showed DNA fibrils in the mitochondrial stroma (see Figure 9.20). Later Nass *et al.* (1965) demonstrated a similar condition to exist in mitochondria from a broad spectrum of biological material. Further details of this nucleic acid and the rest of the mitochondrial genetic system are given in connection with replication of the organelle (Section 9.3.2.).

Dark Granules. Frequently, in mitochondria relatively large, dense granules are present, averaging about three per organelle. Their major constituents had long been reputed to be calcium, inorganic phosphate, magnesium, and carbonate, along with organic material, until they were reinvestigated by Pasquali-Ronchetti and colleagues (1969), who employed 2,4-dinitrophenol and electron microscopy. In treated cells the number of dense granules per mitochondrion was found to be reduced to two within 12.5 min and to one by 30 min exposure to the chemical. Because 80% of the calcium present was eliminated from the mitochondrion within 2 min without a corresponding reduction in the number of granules, the investigators concluded that this element can no longer be considered a major constituent. On the other hand, magnesium was found to be associated with the particles, but the relationship remains obscure.

Furthermore, the extensive participation of calcium in these dense granules was negated by use of high-temperature microincineration (Thomas and Greenawalt, 1968), for they were found, unlike calcium phosphate, to be completely volatilized. Moreover, they have been shown to possess osmiophilic properties (André and Marinozzi, 1965; Ashworth *et al.*, 1966), suggesting a lipid constit-

uent to be present. However, the numerous minute granules that are also present, having diameters less than 0.1 μm, have been demonstrated to contain both calcium and phosphorus (Sutfin *et al.*, 1971).

Macromolecular Organization of Metaphytan Mitochondria. In general, macromolecular organization of the higher plant mitochondrion likewise approximates that of the metazoans. Among the similarities are the repeating units of the membrane, complete with stalked, projecting head pieces (Cunningham *et al.*, 1967) and cytochromes (Baker, 1962). Undoubtedly, many of the same enzymes and enzyme systems of animal mitochondria are present in the plant organelle also, but too few studies have been made to establish their actual existence there.

That it is not safe to make too sweeping generalizations is indicated by the widespread lack of transhydrogenases in plant mitochondria (Hackett, 1963). While good exchange of hydrogen occurs between NAD^+ and NADPH, only one-tenth as much occurs between NAD^+ and NADPH (Ragland and Hackett, 1961). Other, more striking differences are known to exist. For example, although an electron-transport chain replete with cytochromes is present, the series, as pointed out earlier, is by no means identical, for cytochrome c_1 is absent, and several of the *b* type have been found.

One other feature is distinctive of plant mitochondria, an ability for NADH to pass through the membranes (Wiskich and Bonner, 1963). It has been suggested (Lieberman and Baker, 1965) that this property either stems from small crevices present in the membranes of isolated mitochondria despite their seeming intactness, or is perhaps a real capability arising from actual differences in physical or biochemical composition. It might eventually prove to derive from the porosity of the outer membrane described in an earlier section.

9.3. DNA AND MITOCHONDRIAL REPLICATION

Since the discovery of DNA in the mitochondrion mentioned earlier, that substance from this organelle has been investigated extensively as to its physicochemical properties, role in replication, and possible genetic functions. Consequently, the body of literature on this subject has increased to voluminous proportions, including several reviews (Nass *et al.*, 1965; Nass, 1969; Borst, 1972, 1974; Lloyd, 1974; Birky, 1976; Milner, 1976; Borst *et al.*, 1977). As a rule, several DNA molecules are present per mitochondrion, the primary structures of all of which are reportedly similar or nearly identical (Borst, 1972). They are double-stranded, covalently closed molecules, often circular and in most cases collectively comprising about 1% of the total DNA of the Cell.

9.3.1. General Properties of Mitochondrial DNA

Because the physical properties of mitochondrial DNA (mtDNA) have been employed in support of a theory regarding the phylogenetic origins of the

mitochondrion, it is necessary to devote more attention to that biochemical than might otherwise be desirable. While much has been accomplished on the comparative facets of mtDNA structural characteristics, some of its organizational properties have decreased in clarity as knowledge has increased, as becomes apparent almost immediately.

mtDNA in Yeast. Among the protistans, the mtDNA of yeast has been investigated especially thoroughly, so that a number of physical maps of the genome have been published (Groot-Obbink *et al.*, 1976; Heyting and Sanders, 1976; Nagley *et al.*, 1976; Rabinowitz *et al.*, 1976; Sanders *et al.*, 1976a; Schweyen *et al.*, 1976, 1977; Tzagoloff *et al.*, 1976; Borst *et al.*, 1977; Grivell and Moorman, 1977; Grosch *et al.*, 1977; Jacq *et al.*, 1977; Lewin *et al.*, 1977; Van Ommen and Groot, 1977; Linnane and Nagley, 1978). In diploid specimens, the molecule was usually considered to be a circular duplex about 25 μm in circumference, having a molecular weight of 4.5×10^7, equivalent to around 70,000 base pairs (Hollenberg *et al.*, 1970). A study of the mtDNA at various phases of the growth curve, however, has presented a different picture of the structure. Far from being uniformly circular, four Gaussian subpopulations of linear molecules were found and quantified, having mean lengths of 2.2, 4.0, 6.0, and 10.0 μm (Guérineau *et al.*, 1975). With them were found open circular molecules whose circumferences ranged from 0.5 to 10.0 μm. During the exponential growth phase, only 5% of the total DNA molecules were circular and their mean length was 2.2 μm, with a range to 15 μm. With the advent of the transitional phase, circularity increased to 15% and the mean length of the linear forms became 4.4 μm, with a range to 20 μm. Later in the transitional phase, these trends continued, until circular molecules comprised 20% of the total and the greatest length of linear units reached 25 μm. However, in the stationary phase, the circular mtDNA disappeared entirely and the length of the linear forms decreased once more to a mean of 2.2 μm. Furthermore, application of other techniques revealed that the mtDNA became rearranged during the exponential phase, as the mitochondria were differentiated, sometimes forming molecules 40 μm in length (Guérineau and Paoletti, 1975). The reported linearity is in direct conflict with the circularity depicted in all genetical maps of the yeast DNA. It is pertinent to note that in mutants that lack mtDNA, the mitochondria are devoid of cristae (Figure 9.19A–C; Montisano and James, 1979).

When cleaved with restriction endonucleases, the molecule was broken into about 100 fragments, the size distribution of which varied greatly from one strain of *S. cerevisiae* to another. Much of this variability has been attributed to the presence of numerous insertions and/or deletions, many of which were short sections 25 to 50 base pairs in length (Sanders *et al.*, 1977). In addition, in some strains several large insertions between 900 and 1500 base pairs, or even as much as 2600 to 3000 base pairs, in length were present (Sanders *et al.*, 1976b, 1977). For instance, the single gene for the large rRNA has been shown to contain one insert (Bos *et al.*, 1978), while that for cytochrome *b* includes at

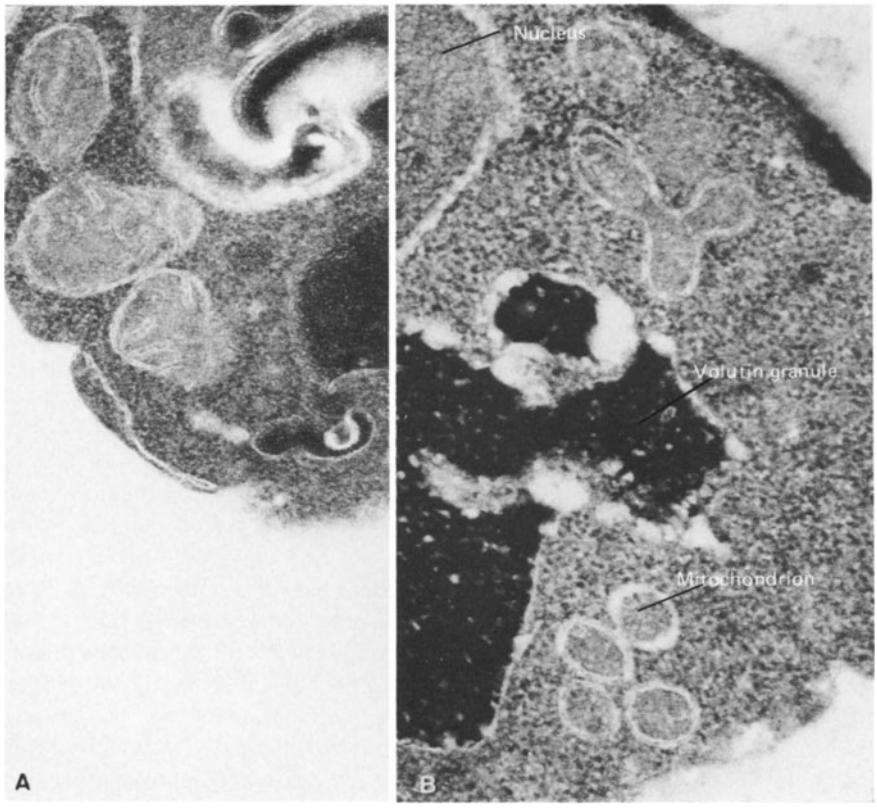


Figure 9.19. Effects of mtDNA on mitochondrial structure. In yeast mutants (*rho*°) that lack mtDNA, the mitochondria (B) lack the cristae found in those of the wild-type specimens (A). (A) 70,000×; (B) 66,000×. (Both courtesy of Montisano and James, 1979.)

least four intervening sections (Borst and Grivell, 1978; Grivell *et al.*, 1979). This condition contrasts strongly with prokaryotes in which no interrupted genes appear to occur.

Comparisons have been made of the sequence homologies of nuclear and mtDNAs of four different species of yeast, *S. cerevisiae, S. carlsbergensis, Candida utilis,* and *Kluyveromyces lactis.* Both nuclear and mitochondrial DNA showed around 90% homology to exist between the two species of *Saccharomyces,* but homologies between other combinations of the four were low, being mostly in the range of 10% (Groot *et al.*, 1975). Moreover, it was demonstrated that the conserved regions of the mtDNAs had acquired several times as many mutated sites than had the nuclear DNA. As a whole the regions that formed DNA · DNA hybrids represented areas that encoded rRNAs and per-

haps tRNAs, those of the nuclear genome being more stable than those of the mitochondria.

With a G + C content of 18 mole %, yeast mtDNA has one of the most A + T-rich genomes known to occur in nature (Bernardi *et al.*, 1970, 1976). The latter reference presented some evidence that was in harmony with considering the A + T-rich segments as spacers, the 70 copies present totaling nearly 50% of the DNA. Some of these segments appeared to be palindromes about 47 nucleotide residues in length and proved to be identical in two strains (Cosson and Tzagoloff, 1979). Of the latter, 24 coded for tRNAs, 2 for rRNAs, and 7 for polypeptides, while the rest remained undetermined (Casey *et al.*, 1972; Reijnders and Borst, 1972; Reijnders *et al.*, 1972; Schatz and Mason, 1974).

One of the strange genetic properties of *S. cerevisiae* is that it has proven relatively easy to isolate mutant forms involving changes in the mtDNA (Williamson *et al.*, 1977). Ease of mutation usually implies the existence of a low ploidy, that is, a relatively small number of genetically active copies of the genome. However, each mitochondrion contains several mtDNA molecules, and every cell encloses perhaps 20 or more mitochondria. Although these organelles seem to be bound together into a single system, around 50 or more DNA molecules would appear to be the minimum number in any given yeast cell. Whereas Williamson *et al.* (1977) give a figure of 44 to 67 for the haploid cell, Grimes *et al.* (1974) found only 10 or 11 in haploid and 22 in diploid specimens. As each molecule apparently is equally capable of being replicated, it would seem virtually impossible for a mutation to segregate out and result in a homoplastic clone, yet, as stated before, segregation occurs rapidly.

Although no adequate explanation has been forthcoming to date, the eventual explanation may involve an organizational unit of the genome that has recently been uncovered (Williamson and Fennell, 1975; Williamson *et al.*, 1977). Through use of a fluorescent dye specific for DNA, it was found that the DNA of yeast mitochondria rarely existed separately but was clustered into aggregates called chondriolites, each containing from one to eight mtDNA molecules (Williamson, 1976). On this basis, each bud might inherit just three to five chondriolites, thus greatly enhancing the possibility of segregating mutants. The actual unit of inheritance in these cells, however, has not been established, nor is it known whether all the DNA molecules in a chondriolite are identical in genetic makeup.

mtDNA in Unicellular Protistans. The mtDNAs from a small number of unicellular protistans have been at least partially characterized. Like some of the yeast types, the double-stranded molecule from *Euglena gracilis* is linear and quite short, having a mean length of between 0.9 and 1.0 μm and a molecular weight of only 1.9×10^6 (Edelman *et al.*, 1966; Krawiec and Eisenstadt, 1970a,b; Manning *et al.*, 1971; Nass *et al.*, 1974). The last report cited also described the presence of a small number of equally small but circular mole-

cules. This smallest of all known eukaryotic genomes has a G + C content of 25% (Fonty *et al.*, 1975). A + T-rich spacers like those of yeast were also reported.

A somewhat greater depth of information has been garnered for *Tetrahymena pyriformis,* whose mitochondria also contain linear DNA molecules (Suyama and Muira, 1968). The latter were found to have a mean length of 17.6 μm corresponding to a molecular weight of 34×10^6 (Suyama and Preer, 1965; Brunk and Hanawalt, 1969). About eight molecules are contained in each mitochondrion (Suyama and Muira, 1968; Flavell and Jones, 1970), all of which have extremely similar primary structures and consist of around 26% G + C (Flavell and Jones, 1971a). Like adenovirus DNA, the linear molecules have been shown to have a terminal duplication, so that extracted molecules that were not renatured formed hairpin structures (Garon *et al.*, 1972; Wolfson and Dressler, 1972; Goldbach *et al.*, 1976), but in contrast to that virus, the duplicated-inversion sector in this ciliate was extremely long, containing about 3500 sites and constituting about 8% of the entire molecule (Figure 9.20). Comparisons of the mtDNAs from five different strains of the organism indicated close kinships to exist only between strains T and ST, which showed about 90% homology (Goldbach *et al.*, 1976). In contrast, homologies between other strains ranged from only 4% to a maximum of 16%. However, all had the terminal duplication-inversion and ragged duplex ends (Arnberg *et al.*, 1977; Goldbach *et al.*, 1977). *Paramecium aurelia* differs strongly from the preceding ciliate in the character of this molecule. Its linear duplex molecule, about 14 μm in length, weighed around 35×10^6 daltons and consisted of 40% G + C (Suyama and Preer, 1965; Flavell and Jones, 1971b; Goddard and Cummings, 1975; Cummings *et al.* 1976). The structure of the mtDNA from *Acanthamoeba castellanii* has been investigated independently by two laboratories (Bohnert, 1973; Bohnert and Herrmann, 1974; Hettiarachchy and Jones, 1974). Approximately 80% of the molecules were circular, with a circumference of 12.7 μm and a molecular weight of 25.7×10^6. Close to 30% of the base composition was attributed to G + C.

An unusual DNase-sensitive structure has been found in the mitochondria of those cells of red algae that give rise to carpospores (Tripodi *et al.*, 1972). Instead of the fibrils that usually characterize the DNA of this organelle, many mitochondria of *Polysiphonia* were in the form of a twisted, complex body (Figure 9.21). This had the appearance of consisting of two series of parallel tubules, the opposing sets being irregularly connected by long fibrils. Similar bodies have been reported from rat hepatocytes and corpus striatum cells and slime mold (Mugnaini, 1964; Behnke, 1965; Schuster, 1965; Blecher, 1967).

Kinetoplast DNA. From other unicellular organisms, little further information regarding this molecule of the mitochondrion seems to have been garnered, except that of the kinetoplast of hemoflagellates, which has been relatively thoroughly explored (Borst *et al.*, 1976; Vickerman, 1977). It has been

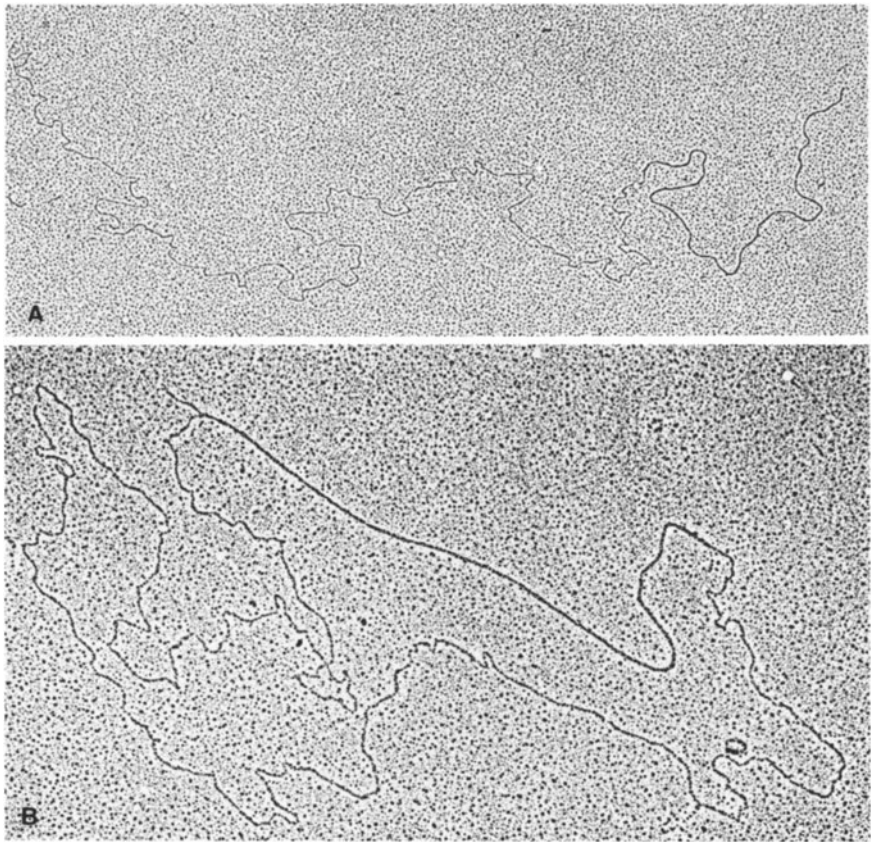


Figure 9.20. Mitochondrial DNA of *Tetrahymena*. The DNA molecule of the mitochondria of this ciliate occurs both as linear (A) and circular (B) forms. (A) 30,000×; (B) 42,000×. (Courtesy of Arnberg *et al.*, 1977.)

found to be quite a complex mixture, four types usually being present (Riou and Delain, 1969; Renger and Wolstenholme, 1970, 1971; Simpson and da Silva, 1971). The greatest quantity was concentrated in the first class (1) consisting of large, apparently linear molecules, whose weight of 43×10^6 resembled that of other unicellular forms (Laurent and Steinert, 1970). Another fairly abundant class (2) consisted of small figure-of-eight molecules plus minicircles catenated with one another. In *Leishmania tarentolae, Trypanosoma cruzi,* and *Crithidia fasciculata,* these had molecular weights of 0.56, 0.94, and 1.49×10^6, respectively (Newton, 1967, 1968; Riou and Paoletti, 1967; Riou and Delain, 1969; Riou and Pautrizel, 1969; Simpson and da Silva, 1971). The

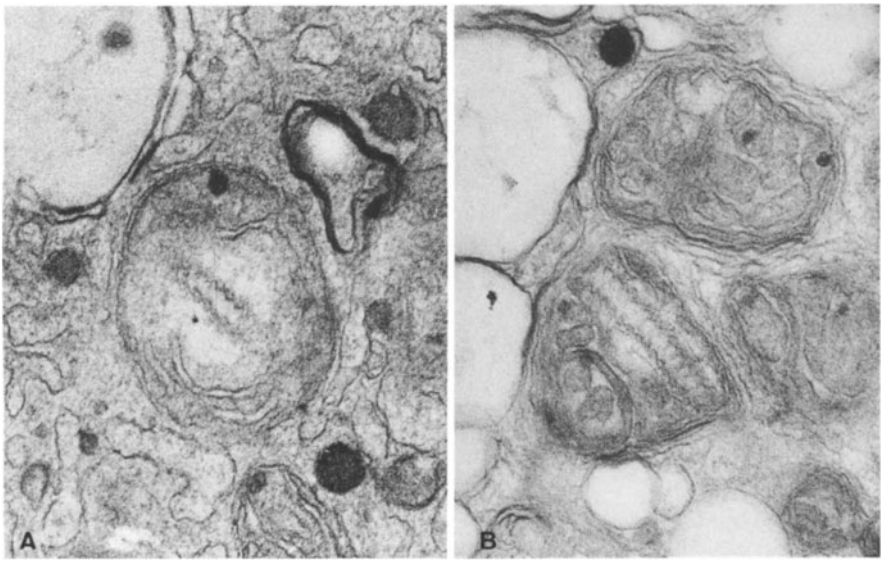


Figure 9.21. DNase-sensitive bodies in *Polysiphonia* mitochondria. (A and B) Such complexes are destroyed by treatment with DNase. (A) 60,000×; (B) 54,000×. (Courtesy of Tripodi *et al.*, 1972.)

minicircles have proven to be highly heterogeneous in base sequence (Kleisen *et al.*, 1976a; Price *et al.*, 1976; Riou and Yot, 1977) and in many forms are linked by thousands in an intricate network of two types (Englund *et al.*, 1977). In form I all the minicircles are covalently closed while in form II they are open. The final two classes were comprised of (3) short, catenated minicircles and (4) individual minicircular molecules. Hybridization investigations uncovered only 2 to 4% homology between the pooled mtDNAs of *L. tarentolae* and *T. cruzi* (Steinert *et al.*, 1973; Lloyd, 1974). More recent analyses have tended to show that a large circular form probably is the true DNA of intact kinetoplast mitochondria (Kleisen *et al.*, 1976b). In addition, two RNA components have been found associated with the DNA in the organelle (Nichols and Cross, 1977).

mtDNA of Fungi. The characteristics of the mtDNA of a small number of fungal types have now been described. That substance from *Allomyces macrogynus* behaved as a single species and proved to have a molecular weight of 52×10^6 and a Gp + pCp content of 42% (Dizikes and Burke, 1978); the configuration of the molecule was not determined, however. *Neurospora crassa* originally was reported to have linear mtDNA molecules 25 μm long and a molecular weight of 40×10^6 (Wood and Luck, 1969; Schäfer *et al.*, 1971; Clayton and Brambl, 1972), with a G + C content close to 40% (Richter and Lip-

mann, 1970). Because both the reported linearity and the homogeneity of the native molecule appeared questionable, further investigations were conducted using improved techniques; these obtained large closed circular molecules (Clayton and Brambl, 1972) and minicircles were also detected (Agsteribbe *et al.*, 1972). The closed circular configuration of the molecule is now well established, as is also a circumference of 19 μm (Terpstra *et al.*, 1977). Moreover, several genetic maps of the mtDNA have appeared in print (Bernard and Küntzel, 1976; Terpstra *et al.*, 1976).

Data available from other fungal sources similarly are incomplete or less than satisfactory. The mtDNAs of *Aspergillus nidulans* and *Kluyveromyces lactis* had molecular weights in the range of 20 to 26×10^6 (Sanders *et al.*, 1974; Lopez Perez and Turner, 1975). This substance from the mitochondria of *Physarum polycephalum* consisted of heterogeneous linear molecules with molecular weights between 40 and 50×10^6 and lengths up to 28 μm (Sonenshein and Holt, 1968; Kessler, 1969); however, more extensive investigations possibly would show that the molecule is actually circular as in *Neurospora*. A base composition including 26% G + C has been reported (Guttes *et al.*, 1967; Evans and Suskind, 1971), while that of the mtDNA from *Dictyostelium* was determined as being 28% G + C (Sussman and Rayner, 1971).

At least a large amount, but not all, of the mtDNA of fungi is concentrated into the so-called mitochondrial nucleus, or better, nucleoid, which is Feulgen positive and undergoes binary fission in a manner reminiscent of the prokaryotic body of the same name (Figure 9.10; Schuster, 1965; Guttes *et al.*, 1966, 1969; Kuroiwa, 1973, 1974; Kuroiwa *et al.*, 1976b). In addition to DNA, these bodies contain RNA, which they synthesize, and proteins, at least one of which is basic (Kuroiwa *et al.*, 1976a). During its synthesis the RNA is distributed nonrandomly on the nucleoid. A somewhat similar body of DNA has been reported in *Polysiphonia,* a red seaweed (Tripodi *et al.*, 1972); this differs strikingly from the fungal nucleoid in having a coiled construction (Figure 9.21).

mtDNA in Higher Plants and Metazoans. In the metaphytans the mtDNA, with lengths up to 62 μm, appears to be the largest reported (Wolstenholme and Gross, 1968; Borst and Kroon, 1969). Quite to the contrary, the molecules from metazoans of all sorts are the very smallest, except for the euglenoids, the circular supercoiled molecule having a mean circumference of 5 μm and a molecular weight of 10×10^6, which corresponds to between 15,000 and 18,000 base pairs (Borst, 1972; Dawid *et al.*, 1976). The actual known range in length is from 4.45 μm in a sea urchin to 5.85 μm in the echiuroid worm, *Urechis caupo* (Dawid and Brown, 1970), and 6.2 μm in *Drosophila melanogaster* (Fauron and Wolstenholme, 1976). Included in the products coded by the molecule are two rRNAs, a set of tRNAs, and a few poly(A)-containing RNAs that appear to be messengers for mitochondrial proteins (Hirsch *et al.*, 1974; Ojala and Attardi, 1974). Apparently the mtDNA molecules of at least mouse and HeLa cells are not pure DNA but contain a fraction of RNA,

for they were nicked when treated with a ribonuclease (Grossman *et al.*, 1973). The major fraction seemingly contained perhaps ten ribonucleotide residues.

Genetic maps of the mtDNA from several vertebrates have been published, including those of two species of *Xenopus* (Dawid *et al.*, 1976), rat liver (Saccone *et al.*, 1976, 1977), and mouse LA 9 cells (Moore *et al.*, 1977; Parker and Watson, 1977). Some years ago, it was reported that the genome coded for only nine peptides, supposedly of the respiratory enzyme complex (Ashwell and Work, 1970; Borst, 1972; Schatz and Mason, 1974), but the latter supposition may have been premature. Unusual heterogeneity in primary structure has been brought to light by a recent investigation that compared different tissues from the same animal and also like tissues from different individuals of the same species, the ox (Coote *et al.*, 1979). Significant differences were found both between liver and brain cell mtDNA and between different specimens. Consequently, the employment of this substance to establish evolutionary histories of a given taxon, as has been done with parthenogenetic lizards of the genus *Cnemidophorus* (Schoen *et al.*, 1979), can scarcely carry great conviction.

The molecular form of the mtDNA of metazoans is also proving to be more heterogeneous than originally suspected (Hayashi *et al.*, 1978a,b). For a period, forms other than monomeric circles were believed to characterize genetically or physiologically abnormal cells, such as cultured tissues, virus-transformed cells, and pathological tissue (Matsumoto *et al.*, 1976). But now, in apparently normal tissues from larvae of three species of *Drosophila,* dimeric and oligomeric circular forms have been found in high proportions, which proved to be head-to-tail concatemers (Shah and Langley, 1977). The number of mtDNA molecules per cell also are variable. Mouse L cells were demonstrated to have 1100 copies per cell of a 1×10^7-dalton molecule, whereas others had 900 copies of a 2×10^7-dalton molecule, and HeLa cells contained about four times as many molecules per cell (Bogenhagen and Clayton, 1974). Moreover, while individuals possessed only one type, two kinds of mtDNA were shown to exist in populations of horses, rats, and man; one of these in the rat proved to have a small core complexed to it (Francisco *et al.*, 1977). Perhaps this is a remnant of a former larger core similar to that of fungi.

9.3.2. Replication and Transcription

The activities of the mitochondrial genetic system at the molecular level are just beginning to be understood to some degree, so any account of the processes must necessarily be brief and greatly incomplete (Young and Hunter, 1979). However, what has been learned reveals differences and similarities to those of both prokaryotes and eukaryotes.

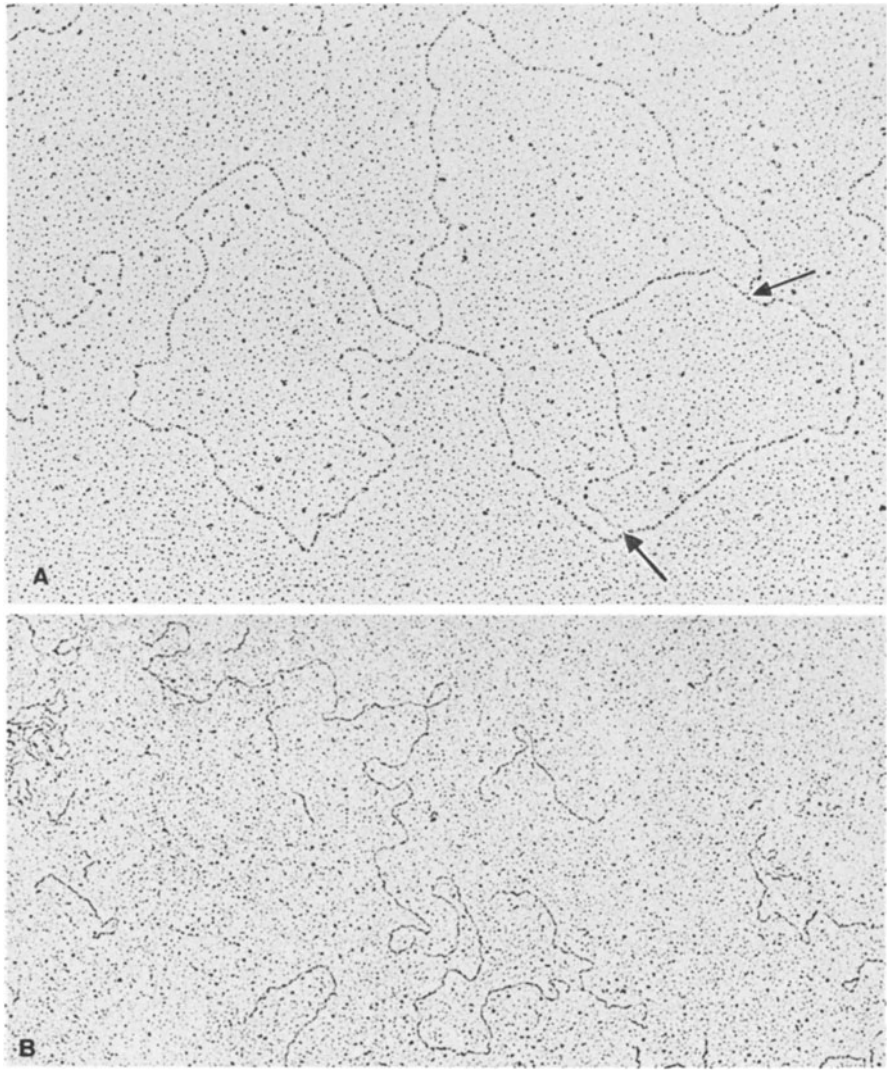
Replication of mtDNA. That DNA was synthesized within the mitochondrion was first established by Parsons and Simpson (1967) who showed

that it required the presence of all four deoxyribonucleotide triphosphates and Mg^{2+}, as in the nuclear processes. However, it was not clear whether this was a replicatory or repair activity until Karol and Simpson (1968) demonstrated that long segments of DNA are synthesized within the organelle, strongly suggestive of replication. This has been further confirmed by use of hybrid mouse–human cells, analyses of which provided evidence that both parental types of mtDNA were synthesized (Coon *et al.*, 1973); such cells have also displayed recombination in producing differently labeled RNAs (Horak *et al.*, 1974). As in viral, prokaryotic, and nuclear DNA of eukaryotes, mtDNA replication involves a membrane–DNA complex (Shearman and Kalf, 1977) and is a discontinuous process (Koike and Wolstenholme, 1974).

The replicative intermediate molecules have been indicated to fall into two contrasting categories. The first class was comprised of branched circles (Figure 9.22), the two branches of which were of equal length (Kirschner *et al.*, 1968; Tobler and Gut, 1974), while the second consisted of closed circular duplexes to each of which was attached a short, single branch, called the E strand (Kasamatsu *et al.*, 1971; Ter Schegget and Borst, 1971a,b). This branch was hydrogen-bonded to the duplex, displacing one of the strands to form a D loop (displacement loop), and could be melted off without breaking the circular portion. As it hybridized exclusively to the L strand of the mtDNA, it probably represented a fragment of the H strand. Both in chick liver mtDNA and that of thyroid, the length of the D loop was 3.5% of the total molecular length (Arnberg *et al.*, 1971). In mouse cells about 50% of the total mtDNA (Kasamatsu *et al.*, 1971; Berk and Clayton, 1976), and in chick liver about 30% (Arnberg *et al.*, 1971), consisted of D loops.

DNA polymerases are present in mitochondria (Soriano *et al.*, 1974; Philippe and Chevaillier, 1978), but little else is known about them. One in yeast mitochondria was found to differ from that of the nucleus in molecular size (Iwashima and Rabinowitz, 1969; Cottrell *et al.*, 1973). At least one of these enzymes is active in repair synthesis, as shown by mitochondria of *Tetrahymena* damaged by ultraviolet light (Westergaard and Pearlman, 1969; Westergaard, 1970; Keiding and Westergaard, 1971). The data of these investigations suggested that repair required concurrent RNA synthesis and that the polymerase was synthesized on cytoplasmic ribosomes with mRNA transcribed from nuclear DNA. However, at least one DNA polymerase has been found to be localized in the mitochondrion (Tanaka and Koike, 1978), one that has proven to be a high-molecular-weight protein, sedimenting at 9.2 S and using poly(A) · oligo(dT) as a template primer (Tanaka and Koike, 1977). Thus in some schemes it may be classified as a DNA polymerase-γ (Weissbach *et al.*, 1975), but the terminology for this class of enzymes has still not become stabilized (Dillon, 1978).

Replication of the mtDNA in the two ciliates that have been investigated shows a number of differences, not only from other eukaryotes but also from



one another. Synthesis of the 15-μm linear duplex molecule of *Tetrahymena* was apparently initiated at the center of the molecule and proceeded bidirectionally from that point toward both ends (Figure 9.23; Arnberg *et al.*, 1974; Clegg *et al.*, 1974; Upholt and Borst, 1974). In the end these processes resulted in the formation of "eye molecules" that later were processed directly into two molecules like the original one. Although the mtDNA of *Paramecium aurelia* was similarly linear and nearly equal in length (14 μm), its replication was shown to proceed unidirectionally from one end, resulting in lariat molecules and linear dimers (Goddard and Cummings, 1975). In the lariat forms, it was

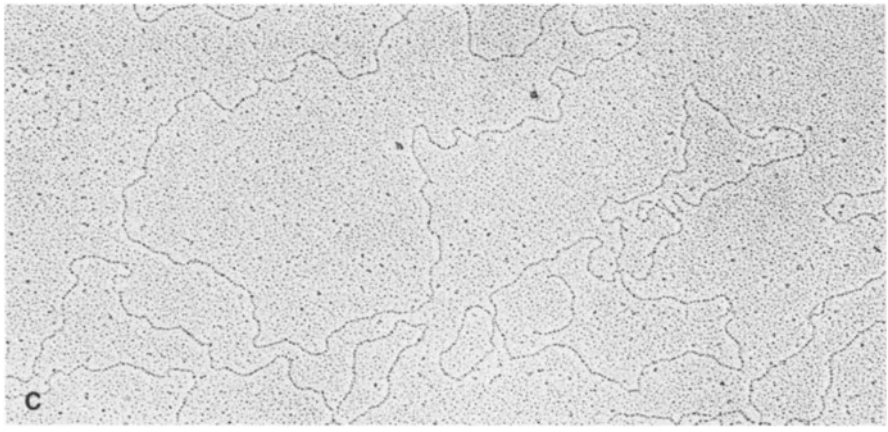


Figure 9.22. Replicative forms of mtDNA from *Ascaris* embryos. (A) Double-forked circular molecule from four-cell-stage embryo; the two segments between the fork (arrows) are of equal length. 96,000×. (B) The heavy band mtDNA from the same source contains linear, unbranched molecules of varied lengths. 20,000×. (C) The light satellite molecules are circular. 60,000×. (All courtesy of Tobler and Gut, 1974.)

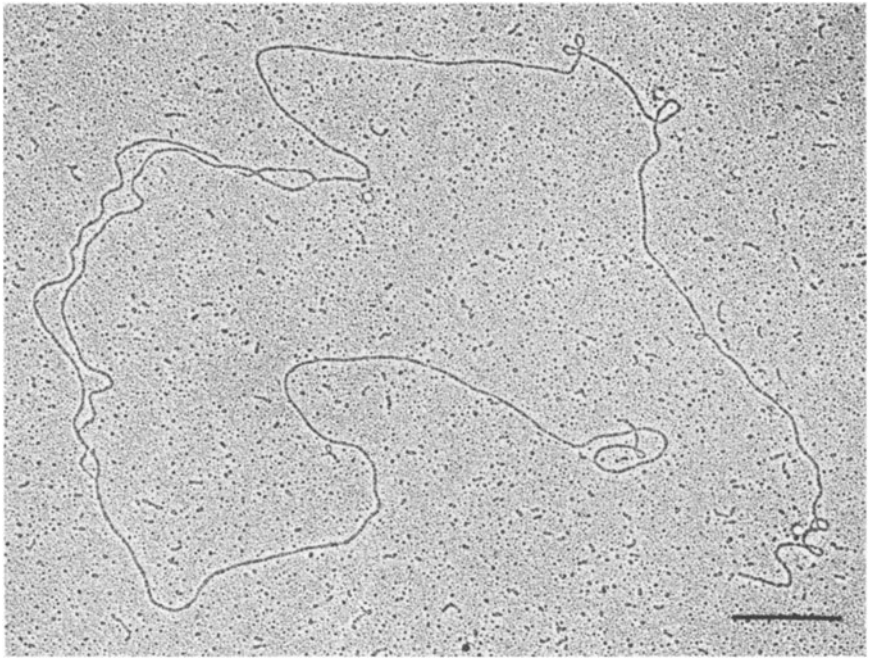


Figure 9.23. A replicating mtDNA molecule from *Tetrahymena*. The linear molecules are replicated bidirectionally from a central point. 48,000×. (Courtesy of Goldbach *et al.*, 1977.)

found that one-half the circumference of the loop plus the length of the tail was consistently equal to 14 μm. It was later demonstrated that the lariats and dimers were true replicative intermediates, the latter being formed directly from the former (Goddard and Cummings, 1977). The dimers were in a head-to-head configuration, each monomer containing a covalent crosslink at the initiation end of the molecule that was retained throughout the processes of replication.

Timing of Replication. Investigations into the correlation of mtDNA synthesis with specific phases of the cell cycle have met with varying degrees of success. In yeasts, mtDNA replication appeared to follow nuclear DNA synthesis and was completed before bud formation began (Smith *et al.,* 1968; Cottrell and Avers, 1970). The second paper cited also detected evidence of synchrony of mitochondrial DNA replication in synchronized cell cultures. Among protistans, *Tetrahymena pyriformis* and *Physarum polycephalum* have been found to replicate mtDNA throughout the cell cycle (Parsons, 1965; Guttes *et al.,* 1967; Charret and André, 1968; Braun and Evans, 1969). In mammals, results are somewhat conflicting, depending on how the cells were synchronized. The Chinese hamster ovary cells synchronized by mitotic selection showed evidence of mtDNA replication after 4 hr, which processes continued for 9 hr, thus resembling nuclear DNA (Ley and Murphy, 1973). Contrastingly, when the cells were synchronized by isoleucine deprivation, mtDNA production did not begin until 9 to 12 hrs after addition of isoleucine but was completed within 3 hr.

Transcription of mtDNA. The DNA of mitochondria is transcribed into various RNAs by specific DNA-directed RNA polymerases (Tzagoloff, 1977) that are distinct from those of both the eukaryotic nucleus and the prokaryotes. In the first place, these enzymes differ in consisting of only a single subunit, and second, unlike the high-molecular-weight complexes of the bacterial and nuclear enzymes, they are relatively small. That of yeast has been reported to be in the range of 59,000 to 67,000 (Rogall and Wintersberger, 1974; Scragg, 1974), that of *Neurospora,* 63,000 (Küntzel and Schäfer, 1971), *Xenopus,* 46,000 to 50,000 (Wu and Dawid, 1972), and rat liver, 65,000 to 70,000 (Reid and Parsons, 1971; Mukerjee and Goldfeder, 1973; Gallerani and Saccone, 1974). In contrast, the RNA polymerase of *Lactobacillus* has been determined as having a molecular weight of 425,000 (Stelter and Zillig, 1974) and that of *E. coli,* 495,000 (Matsura, 1973). The enzyme from both these latter sources had a subunit composition of $\beta\beta'\alpha_2\sigma$ (Travers and Burgess, 1967). Third, while some of the mitochondrial polymerases resembled those of prokaryotes in being sensitive to rifampicin (Reid and Parsons, 1971; Gallerani and Saccone, 1974; Scragg, 1974), those of yeast and *Xenopus* mitochondria were not (Wu and Dawid, 1972, 1974; Rogall and Wintersberger, 1974). At least rat mtDNA-dependent RNA polymerase, quite unexpectedly, has proven to be as sensitive to cycloheximide, a chemical frequently employed to suppress cytoplasmic protein synthesis in eukaryotes (Saccone and Quagliariello, 1975). That of yeast

has recently been shown to be transcribed from nuclear DNA and translated on cytoplasmic ribosomes (Scragg, 1976).

One of the most unusual features of transcription of the mtDNA is that both strands are transcribed completely, producing RNA that hybridizes along the entire lengths of both the H and L chains of the DNA (Saccone and Quagliariello, 1975). Consequently, the immediate product of transcription must be processed extensively to bring the functional molecules into being. Further, this method of transcription precludes the existence of a control mechanism, for one unit of every ultimate product is produced by each cycle of transcription.

Processing the Transcripts. Processing of the single transcript is poorly understood, but it is known to differ from those activities of the cytoplasm and prokaryotes. First, the precursor molecule appears to be cleaved into several large segments, many of which are then polyadenylated on their 3′ termini (Saccone and Quagliariello, 1975). Some of the bases are modified (Borst, 1972; Mahler and Raff, 1975), but whether this occurs before or after polyadenylation remains unestablished. The most distinctive features of processing concern the addition of the poly(A) segments. Like hnRNA, polyadenylation of the nascent mtRNA proceeds rapidly, but here large sectors are recipients. For instance, large chains have been identified that include rRNA in hamster mitochondria; one polyadenylated RNA molecule that sedimented at 20 S has been identified as the precursor for the 17 S RNA of the mitochondrial ribosomes (Attardi *et al.*, 1976; Cleaves *et al.*, 1976; O'Brien, 1977).

Not all the large cleavage products are polyadenylated, however; Attardi *et al.* (1976) separated 18 size classes of poly(A)-containing transcripts and 14 lacking such appendages. In *Neurospora,* to a limited extent a more precise picture of cleavage has been provided, in that it has been shown that a 32 S precursor was cleaved into two smaller precursors. When further processed, the latter ultimately resulted in the mature 25 S and 19 S RNAs of the mitochondrial ribosomes (Saccone and Quagliariello, 1975). Moreover, yeast transcripts as a whole appear to be deficient in poly(A) segments.

In vertebrates, the poly(A) segments have a gel electrophoretic mobility around 4 S, corresponding to a length of between 50 to 80 nucleotides (Perlman *et al.*, 1973). Mouse ascites cell transcripts contained poly(A) sequences only 35 to 55 nucleotides in length (Avadhani, 1979). On the basis of the decay rate, these fell into two classes, one having a half-life of 45 min, the other, 210. In the germinating conidia of the fungus *Trichoderma viride,* the transcripts of the cytoplasmic and mitochondrial fractions were compared directly (Rosen and Edelman, 1976), and it was found that about 10% of the total mitochondrial fraction was polyadenylated. The poly(A)-bearing RNAs of the mitochondria showed only two peaks on polyacrylamide gels, one at 22 S and the other at 29 S, whereas those of the cytoplasm were heterogeneously distributed along the gel. The poly(A) segments of the mitochondrial fraction were only 20 to 25 nucleotides long, while those of the cytoplasm were 50 to 60.

9.3.3. Mitochondrial Ribosomes and Transfer RNAs

The Mitochondrial Ribosomes. The ribosomes of mitochondria have proven to be far less consistent along phylogenetic lines than have their counterparts in the cytoplasm (Table 9.1); hence, it is nearly impossible to make broad generalizations. In the cytoplasm, the monosomic bodies fall with remarkable uniformity into two classes, 70 S in prokaryotes and 80 S in eukaryotes. Quite contrastingly, the monosomic ribosomes of mitochondria range from 55 S in *Paramecium* and rat liver to 78 S in maize and turnip root, and 80 S in *Tetrahymena*. Equally dissimilar results have been noted with the large and small subunits, which show a fair degree of constancy in being 50 S and 30 S in prokaryotes and 60 S and 40 S, respectively, among eukaryotes (Table 9.1). Mitochondrial large subunits, however, range from 39 S in rat liver to 60 S among higher plants, and the small subunit of mitochondria ranges from a low of 30 S in rat liver to a high of 55 S in *Tetrahymena,* in which ciliate the large and small subunits are identical in size (Suyama, 1967; Chi and Suyama, 1970). In addition to these types within the stroma, cytoplasmic ribosomes are bound to the outer surface of the organelle, at least in yeast (Kellems and Buetow, 1974).

The preceding comparisons, disparate as the results are, still do not show the true lack of consistency of the mitochondrial ribosomes. For instance, the RNAs of their subunits differ strongly from one taxon to another, that of the large subunit showing a range from 16 S in rat liver to 23 S in *Neurospora* (Table 9.1), whereas the RNA of the large subunit of the cytoplasmic ribosomes only varies between 25 S and 29 S. Where they have been established, protein numbers likewise show a broad base. This statement is also true of the cytoplasmic particle, which has totals ranging from 25 to 71, but to a lesser degree than the mitochondrial ribosome, where only 20 proteins have been detected in the yeast and as many as 107 in *Neurospora*. Thus it is obvious that the ribosomes of mitochondria have little in common with either the prokaryotic or the eukaryotic cytoplasmic granule.

This distinctiveness is accentuated by the observation that all the proteins of the mitochondrial ribosomes except one (S-5) in *Neurospora* are synthesized by the cytoplasmic organelle (Küntzel, 1969; Neupert *et al.,* 1969; Lizardi and Luck, 1972; Lambowitz *et al.,* 1976, 1979; Buetow and Wood, 1978), and all seem to be thus formed in yeast (Davey *et al.,* 1970; Schmitt, 1970, 1971). In *Tetrahymena,* a few of the very small proteins of the mitochondrial particle are synthesized within that organelle, but even the synthesis of these is under the control of the nuclear system (Millis and Suyama, 1972). A similar statement applies to the mitochondrial ribosome proteins of *Paramecium* (Tait *et al.,* 1976). That the situation is complex has been shown by some investigations on interspecific hybrids of *Xenopus laevis* and *X. mulleri* (Leister and Dawid, 1975). The F_1 hybrids received nuclear genes from both parents, but the mi-

Table 9.1
Comparison of Ribosomal Structures

Source	Mono-some	Large subunit		Small subunit		5 S rRNA	5.8 S rRNA	Number of proteins		
		Total	rRNA	Total	rRNA			Total	Large subunit	Small subunit
Prokaryote										
E. coli	70 S	50 S	23 S	30 S	16 S	Present	Absent	56	34	21
Eukaryotes										
Yeast	80 S	60 S	25 S	40 S	16 S	Present	Present	---	36-55	30-40
Tetrahymena	80 S	60 S	26 S	40 S	17.5 S	Present	Present	25	---	---
Neurospora	77 S	---	25 S	---	17 S	Present	Present	---	---	---
Rat liver	83 S	47 S	32 S	32 S	16 S	Present	Present	---	---	---
Pea	78 S	55 S	---	35 S	---	Present	Present	---	---	---
Turnip	80 S	60 S	25 S	40 S	18 S	Present	Present	---	---	---
Mitochondria										
Yeast	72 S	---	22 S	---	18 S	Present	(13S)	20	---	---
Tetrahymena	80 S	55 S	21 S	55 S	14 S	Present	Absent	---	---	---
Neurospora	73 S	52 S	24 S	39 S	17 S	Absent	Absent	107	---	---
Rat liver	55 S	39 S	16 S	30 S	12 S	Absent	Absent	84(107)	40	44
Turnip	78 S	60 S	---	46 S	---	Present	Absent	---	---	---
Chloroplasts										
Euglena	---	---	22 S	---	16 S	---	---	---	---	---
Chlamydomonas	70 S	60 S	---	45 S	---	---	---	---	---	---
Maize	78 S	60 S	---	44 S	---	Present	Absent	---	---	---

tochondrial genes came solely from the egg. When the proteins of the large subunit of the mitochondrial ribosomes were compared, one species was observed to be absent from each F_1 progeny. The presence of certain others was related to the sex and species of the parent, one being absent if that species of clawed frog had been the mother, and four others if the corresponding species had been the father. One explanation offered was that, although the hybrid nuclear genomes contained genes from both parents, only maternal proteins were actually incorporated into the mitochondrial ribosome. However, this does not explain the presence in one case of the paternal protein.

Unlike the proteins of the mitochondrial ribosomes, the rRNAs are encoded by mtDNA and transcribed and processed within the mitochondrion (Reboul and Vignais, 1974). Little appears to have been ascertained regarding processing, but in hamster cells it includes a low degree of methylation (Dubin, 1974). The 17 S nucleic acid of the large subunit was demonstrated to contain 0.13 methyl group per 100 nucleosides; further analysis showed that one methylated ribose moiety and one unidentified methylated residue were present per molecule. The 13 S RNA of the small subunit was slightly more heavily methylated, having 0.37 methyl group per 100 nucleosides; the modified bases appeared to include one thymidine, one N^6-dimethyladenosine, and one methylated cytidine residue per molecule.

Mitochondrial tRNAs. The mitochondrial genome codes for a variety of tRNA species (Casey *et al.*, 1972, 1974a,b; Cohen and Rabinowitz, 1972), but the precise number encoded is highly diversified among eukaryotes. In *Tetrahymena,* only two types were found to be coded by the mtDNA, those for phenylalanine and leucine; however, three isoaccepting $tRNAs^{Leu}$ were later identified (Chiu *et al.*, 1974). These four that were formed in mtDNA were referred to as "native" tRNA, while others that did not hybridize to mtDNA were considered "imported" from cytoplasmic sources (Chiu *et al.*, 1975). *Neurospora crassa* has been shown to lie at the opposite end of the scale, for its mitochondrion-encoded tRNAs numbered 18 (Barnett and Brown, 1967); this is exceeded by yeast, whose mtDNA coded for 20 tRNAs (Martin *et al.*, 1977), including two $tRNAs^{Met}$. Previously elsewhere, three valine tRNA-isoaccepting species had been reported to be mtDNA products (C. Schneller *et al.*, 1975). The location of the genes for many of these on the mitochondrial genome has been determined (Fukuhara *et al.*, 1976; Martin *et al.*, 1977); some isoaccepting species have been found to be transcribed from different regions of the yeast mitochondrial genome (N. C. Martin *et al.*, 1976). Three distinct $tRNAs^{Ser}$ were shown to be encoded by the mtDNA, while others from the cytoplasm were imported selectively (Baldacci *et al.*, 1976, 1977).

Among mammalian sources, the number of tRNAs varies greatly from species to species (Aujame and Freeman, 1979). In HeLa cells, Lynch and Attardi (1976) detected 17 tRNAs that could hybridize with mtDNA; these specified 16 different amino acids, the four missing species being for asparagine,

glutamine, histidine, and proline. Thus this is the sole source from which a mitochondrial $tRNA^{Cys}$ has been reported. Only six amino acids have been found provided with mitochondrial tRNAs in rat liver (Buck and Nass, 1969; Nass and Buck, 1970; Jakovcic *et al.*, 1975), and an identical number have been described from calf brain (Charezinski and Borkowski, 1973; Borkowski and Brzuszkiewicz-Zarnowski, 1975). Contrastingly, four isoaccepting species of $tRNA^{Met}$ have been identified in mouse liver mitochondria (Wallace and Freeman, 1974b). That the relationship among tRNAs of mammals is limited largely to intraordinal boundaries was indicated by the finding that the mitochondrial $tRNA^{Leu}$ from rat liver would hybridize under stringent conditions only to that mtDNA of mouse and guinea pig, in addition to its own (Jakovcic *et al.*, 1975).

Molecular Traits of Mitochondrial tRNAs. Some years ago molecular weights of mitochondrial tRNAs were reported to be slightly lighter in some cases than those of the corresponding cytosolic types (Dubin and Friend, 1972), but so far this condition has actually been demonstrated only for the $tRNA^{Leu}$ of the hamster (Aujame *et al.*, 1978). In that rodent, two isoaccepting species of leucine from the mitochondria were found to have molecular weights of 23,000 and 24,000, respectively, in contrast to the 27,000 of the same type from the cytosol. Moreover, at least mammalian sources have a lower level of unusual bases. In particular, 7-methylguanosine has been noted to be rare in mitochondrial varieties (Dubin and Friend, 1974; Chia *et al.*, 1976; Wallace *et al.*, 1978), which nucleoside in prokaryotic and cytosolic tRNAs occurs only in site C of the short type arm IV (Dillon, 1978). However, while present there in most species that have been sequenced, it is absent with a high degree of variability from one source to another. This lack of homology between tRNAs of the mitochondria from diverse organisms continues at the gene level, for hybridization experiments with tRNAs and mtDNA from different sources show only low degrees of interaction (Jakovcic et al., 1975).

Primary Structure of $tRNA^{Met}$. The base sequence of one mitochondrial tRNA has been determined, that of the initiator $tRNA^{Met}$ of *Neurospora crassa* (Heckman *et al.*, 1978). Comparison of its primary structure with others of the same tRNA type from other sources (Table 9.2) suggests close relationships with neither those of prokaryotes nor eukaryotes. Although it is formylated for use in initiation as in most prokaryotic but no known eukaryotic tRNAs, not all of the former types are so modified. For instance, *Halobacterium cutirubrum* does not employ formylated $tRNA^{Met}$ for initiation (Heckman *et al.*, 1978). On the other hand, it resembles those of eukaryotic sources in having the bases in sites 1 and 1′ of arm I form a standard Watson–Crick pair, while the initiator species of prokaryotes cannot, *Halobacterium* again being exceptional (Heckman *et al.*, 1978).

In the sequence, arms homologous in composition to those of other sources are scarce. Arm IA (Table 9.2) shows resemblances to eukaryotes as

Table 9.2
Comparison of the Initiator RNA of Neurospora[a]

	Arm IA	Bend 1	Arm II	Bend 2	Arm IIIA	Anticodon
E. coli$_{F1}$	p CGCGGGG	U_tG	GAGC AGCCUGGD--A GCUC	G	UCGGGC$_2^1$U	CAU
N. crassa$_{mt}$	p UGCGGAU	UA	UUGU AA-DAG-D--A ACAU	A	UUUGGCU	CAU
S. cer$_F$	p AGCCGCG	UG_1	G_2CGC AG-D-GG--AA GCGC	G_2^2	CAGGGCU	CAU
Mus$_4$	p GCCUCGU	UA	G_2CGC AG-DAGGD--A GCGC	G_2^2	ΨCAGΨCU	C_2AU

	Arm IIIB	Arm IV	Arm V	Arm IB	Preterm
E. coli$_{F1}$	AACCCGA	AGG_7UG	GUCGG TΨCAAAU CCGGC	CCCCGCA	A
N. crassa$_{mt}$	G_1ΨCCGAA	-UGAC	AUAGG UGCAAAU CCUGU	AUCCGCA	U
S. cer$_F$	A_tACCCUG	AUG_7DC_5	CUCGG AVCGA_1AA CCGA$\underset{m}{G}\underset{m}{}$	CGCGGCU	A
Mus$_4$	A_tAΨCUGA	AGG_7DC_5	GUGAG TΨCGA_1UC CUCAC	ACGGGGC	A

[a]Based on Dillon (1978) and Heckman *et al.* (1978).

well as prokaryotes, while bend 1 is strictly eukaryotic in composition. In arm II, the double-stranded sector displays kinships to neither group, but the single-stranded loop is predominantly eukaryotic in nature, especially in having two dihydrouridine residues present. Bend 2 is totally unique, as is also the double-stranded region of arm III. Also in the latter arm, sites A and B of the unpaired loop correspond to those of eukaryotic species, whereas those of sites C and D are unrelated to any other type. A similar lack of relationships in primary structure is found in the remainder of the molecule, including the preterminal site.

Taken as a whole, the molecule is unusually rich in uridine residues. If the modified species of this nucleoside, such as pseudouridine and dihydrouridine, are included, the count of such residues totals 23, which compares to 11 and 10 for the *E. coli* and yeast initiator tRNA, respectively, and to 15 for the mouse $tRNA_4^{Met}$. Adenosine residues are also more abundant than elsewhere but less strikingly so than uridine, 19 being present here compared to 13 for the bacterial, and 15 for the eukaryotic cytoplasmic types. Finally, a paucity of modified bases can be noted (Wallace *et al.*, 1978), there being only 4 present, contrasting to 6 in the prokaryote, 11 in the yeast, and 15 in the mouse species. Because each modified site requires a separate modifying enzyme, this lack of modification could well be correlated to the reduction of the genome that characterizes the mitochondrion of all eukaryotes.

This loss in turn could also apply to the noted absence (J. Schneller *et al.*,

1975a,b) of the hypermodified base referred to as Y that characterizes the tRNA^{Phe} of many eukaryotes (Dillon, 1978). The sparsity of modified bases and the high frequency of uridine and adenosine have been recorded from such mammalian sources as the mitochondria of rat liver and Morris hepatomas 5123D and 7777 (Chia *et al.*, 1976). Studies on the methylases of yeast showed that, while a large variety of types occurred in the cytoplasm, only those of 1-methyl- and 2-methyl-guanosine, 2-methyl-N_2, N_2-dimethylguanosine, and ribothymidine occurred in the mitochondria (C. Schneller *et al.*, 1975, 1976). Other studies have shown that in addition to the products of these methylases, inosine, pseudouridine, and t^6A also occurred in the mitochondrial tRNAs of yeast (R. Martin *et al.*, 1976).

Mitochondrial tRNA Ligases. The tRNA ligases (or synthetases as they are too frequently called) of mitochondria have been investigated to only a limited extent, but that little has sufficed to reveal a complicated picture, the complexity being derived in large measure from the presence in the mitochondria of native and imported tRNAs. Frequently, the mitochondrial ligases form a class distinct from those of the cytoplasm, as reported for yeast (Boguslawski *et al.*, 1974; Baldacci *et al.*, 1975; J. Schneller *et al.*, 1975c), *Neurospora* (Epler *et al.*, 1970), *Tetrahymena* (Suyama and Eyer, 1967; Chiu and Suyama, 1975), and metazoans (Barnett *et al.*, 1967; Buck and Nass, 1969; Chia *et al*, 1976). For a case in point, all three isoaccepting mitochondrial tRNAs^{Leu} from *Tetrahymena* were reported to be native species that were aminoacylated by a valyl-tRNA ligase that showed no structural relationship to the corresponding enzyme of the cytoplasm (Chiu and Suyama, 1975; Suyama and Hamada, 1976). In contrast, the two isoaccepting tRNAs^{Val} are imported and are aminoacylated in the mitochondrion by a ligase that is indistinguishable from that of the cytoplasm (Suyama and Hamada, 1978). The suggestion has been made that perhaps the ligase plays a key role in the transport of the tRNA across the mitochondrial membrane, but no evidence exists either in support of or in opposition to that proposal (Suyama and Hamada, 1976, 1978). Not all the ligases of specific imported mitochondrial tRNAs are identical to the corresponding enzyme of the cytoplasm, however; those of the four aminoacyl types tested in addition to valine showed chemical properties markedly different from those of the counterparts in the cytoplasm (Suyama and Hamada, 1978, Table 3).

9.3.4. Protein Synthesis in Mitochondria

Mitochondrial Protein Biosynthesis. Mitochondria are capable of synthesizing proteins (Scragg *et al.*, 1971; Stegeman and Hoober, 1974; Innis and Craig, 1978), but maximum rates are more sensitive to ionic composition of the medium than are the cytoplasmic systems. Relatively high concentrations of Mg^{2+} and K^+ cations are especially important (Roodyn *et al.*, 1961; Beattie,

1971; Mockel, 1972); Na^+ or NH_3^+ may be substituted for the K^+, but they do not yield as rapid rates of amino acid incorporation (Grivell *et al.,* 1971). In addition, an energy source, such as ATP or an ATP-generating system, also is essential, along with the other common nucleoside triphosphates. Furthermore, the mitochondrial system has been clearly demonstrated to be completely uncoupled from the nuclear system (Rouslin, 1977).

Initiation of protein synthesis involves an interaction between the small subunit of the mitochondrial ribosomes, mRNA bearing the initiation triplet (AUG), formylated methionyl-$tRNA_F$, GTP, and initiation factor IF-2 as in cytoplasmic systems (Jay and Kaempfer, 1975; Dillon, 1978, p. 163 ff). The requirement for formylated initiator $tRNA^{Met}$ still has some problem areas. First, the source of the formyl radical has not been clearly established, but the most probable donor is N^{10}-formyltetrahydrofolate (Galper and Darnell, 1969; Galper, 1974). When cultured in the presence of methotrexate (a drug that depletes the C_1-folate pools) and metabolites whose synthesis is mediated by such pools, mouse L cells and KB cells grew normally, with no apparent effect on the formylation of the initiator tRNA (Wallace and Freeman, 1974a). Hence, the mechanism through which the initiator tRNA was formylated under these conditions remained obscure, nor could it be discerned whether or not protein synthesis had proceeded normally in the possible absence of formylation. In all systems, the initiation factors are located on the ribosomes, but those of mitochondrial ribosomes are not interchangeable with those of the cytoplasm (Avadhani and Buetow, 1974). Another problem, which has received no attention, is how initiation occurs in *Tetrahymena* in the mitochondria of which ciliate no $tRNA^{Met}$ of any sort, native or imported, formylated or unformylated, has as yet been detected (Suyama and Eyer, 1967; Chiu *et al.,* 1974, 1975; Suyama and Hamada, 1976).

Peptide-chain elongation and termination processes in the mitochondrial system have received little or no attention to date. The elongation factors EF-G and EF-T are known to be present, however, and differ from those of the cytoplasm (Avadhani and Buetow, 1974; Lewis *et al.,* 1976). In contrast, those of *Euglena* are at least partially interchangeable with the corresponding cytoplasmic enzymes (Avadhani and Buetow, 1972a,b).

The quantity of protein synthesis that takes place in mitochondria has been investigated to some degree. In *Chlamydomonas,* the rate and extent of incorporation of labeled leucine into mitochondrial proteins were such that it appeared that all proteins encoded by the mitochondrial genome were probably synthesized in that organelle (Stegeman and Hoober, 1974). In a trypanosomatid, *Crithidia luciliae,* a series of studies presented evidence that perhaps as much as 50% of the protein biosynthesis of the entire cell took place in the single large mitochondrion (Laub-Kupersztejn and Thirion, 1969, 1972, 1974), but other laboratories using the same organisms found that only about 3% of the total actually occurred there (Kleisen and Borst, 1975). Actually more than

90% of the proteins employed by mitochondria are encoded by nuclear DNA and synthesized on cytoplasmic ribosomes (Ashwell and Work 1970; Linnane *et al.*, 1972), but the interrelationships of subunits of large proteins are proving to be complicated. For example, four of the subunits of cytochrome *c* oxidase are synthesized in the cytoplasm and then assembled into the holoenzyme in the mitochondria with three subunits synthesized there (Groot and Poyton, 1975; Poyton and Groot, 1975; Eggitt and Scragg, 1975; Eggitt, 1976). It is possible that subunits of this oxidase are the sole products of mitochondrial protein synthesis in *Neurospora* (Rowe *et al.*, 1974). A number of subunits similar to that of cytochrome *c* oxidase and with corresponding sites of production (three in the mitochondria, four in the cytoplasm) have been reported for the cytochrome bc_1 complex (Katan *et al.*, 1976; Marjana and Ryrie, 1976; Buetow and Wood, 1978). While the details have not become firmed as yet, mitochondrial ATPase appears to have a comparable dual origin (Tzagoloff *et al.*, 1973, 1974; Jackl and Sebald, 1975).

Other Products Synthesized in Mitochondria. Several other classes of substances in addition to proteins and RNAs are produced within the mitochondrion. Some of the minor products are guanosine polyphosphates, such as 5′-diphospho-guanosine-3′-diphosphate (ppGpp) and 5′-triphosphoguanosine-3′-diphosphate (ppGpp) (Horváth *et al.*, 1975). These substances characteristically are placed at one end of RNA molecules in prokaryotes as well as eukaryotes, where they play an unknown role.

Of more importance to the economy of the organelle are the origins of pyridine nucleotides, such as nicotinamide mononucleotide (NMN) and nicotinamide adenine dinucleotide (NAD), to which the inner mitochondrial membrane is nearly impermeable (von Jagow and Klingenberg, 1970). However, nicotinic acid and nicotinamide can penetrate the membrane readily. When labeled, either of these substances could be found incorporated into NMN or NAD within isolated intact or sonically disrupted mitochondria (Grunicke *et al.*, 1975; Lange and Jacobson, 1977). An absolute requirement of ATP, 5-phosphoribosyl-1-pyrophosphate, and $MgCl_2$ was demonstrated.

9.3.5. Mitochondrial Replication

Two major aspects of the biogenesis of mitochondria merit attention, one at the cellular level and the other at the molecular. Viewed as the whole organelle, there are three possible sources for the origin of new mitochondria: (1) other membranous portions of the cell, (2) division of existing mitochondria, and (3) *de novo* synthesis (Berger, 1964; Baxter, 1971; Afzelius, 1972).

Replication at the Cellular Level. As has appeared on more than several occasions in preceding pages, earlier workers occasionally advocated an origin for the mitochondrion from various other cell organelles, including the nuclear

envelope, endoreticulum, and dictyosomes (Novikoff, 1961) and even the plasmalemma (Robertson, 1959). In view of the specialized nature of each of the membranes and the diverse natures of their enzymatic content, there appears to be little justification for such simplistic concepts. Perhaps an organelle of one sort or another could serve as a focal point on which a mitochondrion might be assembled as flagella are, but that is quite another matter and amounts to *de novo* synthesis.

Little doubt persists that the most frequent method of producing more copies of this organelle is from preexisting mitochondria by growth and division. The details of the processes are elusive, however. Indirect evidence of fission was first advanced by Luck (1963), who used labeled choline and a choline-requiring mutant of *Neurospora*. Radioautograms from samples taken at timed intervals following exposure to the radioactive substance revealed a random pattern of labeling in the mitochondria of daughter cells, rather than the nonrandom pattern that would have resulted either by *de novo* synthesis or from preexisting membranous organelles. The fine series of electron micrographs on division of the organelle and cylinder of DNA in fungi reproduced in earlier pages (Figure 9.10) and others from cinematomicrographic investigations cited in relation with mitochondrial motility (Section 9.1.4) leave little room for doubt that one means of reproduction in this organelle is by binary fission (Tandler *et al.*, 1969).

One source of evidence for *de novo* production of mitochondria is from anaerobically grown bakers' yeast. Provided that no ergosterol or unsaturated fatty acids have been added to the culture media, *S. cerevisiae* cells grown in the absence of air contain no trace of mitochondria (Linnane, 1965). When aeration of such organisms continues for about 3 hr, vesicles and cytoplasmic membranes appear, followed in another hour by incomplete mitochondria (Section 9.1.3.), and after 8 hr by complete ones. It should be noted that the anaerobically grown cells did not contain cytochromes *a*, a_3, or *b*, although small amounts of cytochrome *c* were present. However, all the cytochromes were detected after aeration.

In a second set of evidence indicative of *de novo* origins, the events parallel those of anaerobic yeast. The source material, the inner segment of the photoreceptor cells of fish retina, was claimed to be ideal for investigations of developing mitochondria (Figure 9.24), because those organelles become extremely abundant, are contained in a well-defined space, and their genesis occurs in a gradient (Berger, 1964). The steps in development, deduced from serial-sectional analysis of embryonic guppy eyes, began with double-membrane sheets and small vesicles in the cytoplasm. Then the membranes folded around the vesicles, until the ends met and subsequently fused. In the meanwhile the vesicles elongated and compressed to form cristae. After the latter had become more abundant and the entire organelle had increased in size, the definitive mitochondrion was thereby brought into being.

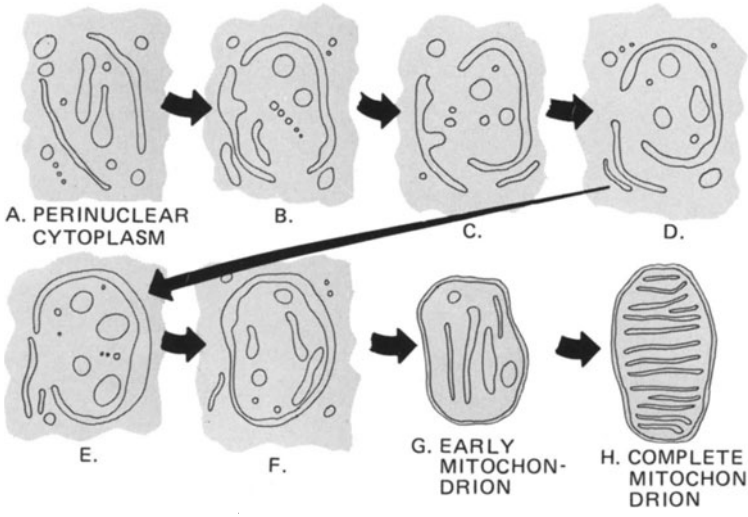


Figure 9.24. Stages in the *de novo* origins of mitochondria in photoreceptor cells. (Based on Berger, 1964.)

Regeneration of Mitochondria. Events in the regeneration of mitochondria in organisms emerging from quiescent stages such as cysts parallel those of the yeast to some extent, except in not being entirely *de novo*. One notable investigation of these processes followed the changes in the organelle subsequent to emergence of *Artemia salina* (the brine shrimp) from the encysted stage (Schmitt *et al.*, 1973, 1974) as follows. When induced to form the cyst by dehydration, embryonic growth ceased at the gastrula stage of development. After being rehydrated, it underwent development to the prenauplius stage, which process took place in 15 hr without cell division. Further incubation then led to hatching, followed by the resumption of cell division and development into the free-swimming nauplius. If oxygen consumption is expressed as $Q_{O_2} = \mu$moles O_2 per hour per gram of dried cysts, an increase from $Q_{O_2} = 11$ to $Q_{O_2} = 27$ occurred within an hour after incubation was initiated at 30°C. This quantity reached a plateau at 32 in the prenauplius and rose to 75 after hatching. These increases were paralleled by increments in cytochrome oxidase activity and also in quantities of cytochromes *b* and *c* present. Concurrent studies of the changes of the mitochondria were also conducted. In the cysts, mitochondria were present but lacked cristae; however, within an hour after incubation was commenced, a few such structures could be noted along the periphery. As development continued, cristae became abundant as the mitochondria attained mature size in the nauplius.

The main event, then, in regeneration seems thus to be the formation of

the cristae, four avenues for their origins being available: They could form (1) as mere outpocketings of the inner membrane or (2) *de novo;* (3) they might represent remnants of the inactive inner membrane, while a new inner investing membrane formed; or (4) both the inner membrane and the cristae could be combinations of new membrane with old remnants. The only known evidence, the differences in enzymatic content of cristal and inner membranes, militates against the first alternative, but no data are available that either support or conflict with the remainder. However, it is not unlikely that the fourth possibility will eventually prove to be the route actually followed.

9.4. MESOSOMES: PRIMITIVE RESPIRATORY ORGANELLES?

A structure found in bacteria nearly three decades ago, while thoroughly explored morphologically, is still controversial in regard to its physiology (Burdett and Rogers, 1972; Greenawalt and Whiteside, 1975). Part of the lack of understanding comes from conflicting results through use of differing experimental procedures, part from the confusion of too many proposals based solely on limited ultrastructural observations, and part from the difficult nature of the organelle itself. As shown by the discussion that follows, discordant reports have characterized the body throughout its known history.

9.4.1. The Bacterial Mesosome

Many years prior to its identification by electron microscopy of the intact organism, a membranous organelle of bacteria, the mesosome (Figure 9.25), had been identified *in situ* by selective staining as a respiratory structure, and later it was isolated by means of cell fractionation and ultracentrifugation. While Mudd and his colleagues (1951a,b, 1956; Mudd, 1954, 1956) were seemingly pinpointing the respiratory organelle by cytochemical methods, Kellenberger and Huber (1953) separated a layer of high respiratory activity from bacteria and showed it to consist of structureless particles. Still later, a number of electron microscopic investigations, such as those by Niklowitz (1958) and Giesbrecht (1960), correlated this particular with a peculiar body in these organisms, often attached to the nucleoid and cytoplasmic membrane.

More recently, combinations of biochemical and ultrastructural procedures comparable to those employed with metazoan cells have revealed active respiratory sites in intact bacteria. The first attempts along these lines were not conclusive. Using 2,3,5-triphenyl tetrazolium, Vanderwinkel and Murray (1962) and Takagi and his associates (1965) demonstrated sites of oxidation–reduction activity but were unable to relate them to regularly occurring, clearly defined organelles. Subsequently, van Iterson and Leene (1964) employed tellurite, and Sedar and Burde (1965) made use of a tetrazolium salt, trinitro blue

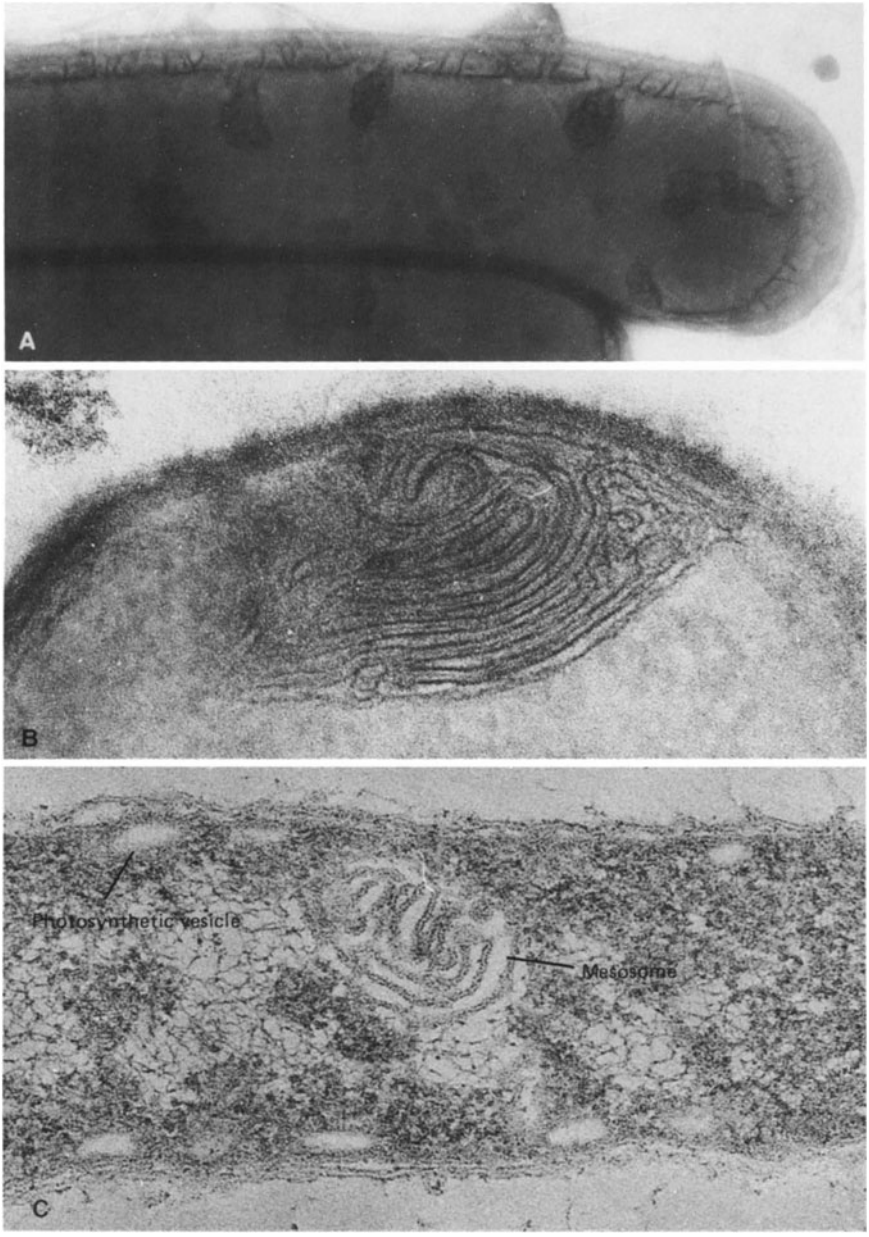


Figure 9.25. Mesosomes of two bacteria. (A) Mesosomes may be distributed throughout the bacterial cell, as in this specimen of *Bacillus licheniformis*. 41,000×. (B) The mesosome of this species when sectioned is seen to display an unusually high degree of complexity. 196,300×. (Both courtesy of Burdett and Rogers, 1972.) (C) The corresponding organelle of *Chlorobium thiosulfatophilum* appears to be less complex and more loosely folded than the foregoing. 120,000×. (Courtesy of Cohen-Bazire *et al.*, 1964.)

tetrazolium, whose reduction product is insoluble in the reagents utilized during tissue preparation. Both teams of investigators were able to demonstrate clearly that the principle center of succinic acid reduction lies in the organelle known as the mesosome. Yet when isolated from the cell, in some laboratories succinic hydrogenase activity was so low as to suggest its total absence (Reaveley and Rogers, 1969), and the same statement is true of other components of the respiratory cycle (Salton and Owen, 1976; Salton, 1978). In contrast, other laboratories reported a full spectrum of the enzymes to be present (Pangborn *et al.*, 1962).

The mesosome, a name first applied by Fitz-James (1960), is variously called also the onion-shaped body, chondrioid (Kellenberger *et al.*, 1958; van Iterson, 1965), and plasmalemmasome (Edwards and Stevens, 1963). By both ordinary and freeze-etch techniques (Remsen, 1966, 1968), the organelle has been shown to consist of a spiral or much-folded membrane, closely associated usually with the plasmalemma and frequently with the nucleoid, as mentioned above (Ryter, 1968). More recent work, with improved techniques, has demonstrated that the membranes are heavily associated with numerous tubules and moreover are often themselves folded into tubulelike structures (Burdett and Rogers, 1972). In many electron micrographs the organelle actually appears to be derived from the cell membrane; indeed, the study on *Diplococcus* by Tomasz and co-workers (1965) leaves little room for doubt as to this derivation. Although the mesosome quite frequently is located toward one end of the bacterium in proximity to, or even upon, the septum, this location is by no means constant, for as reported below, the body actually migrates during the cell's cycle of growth and, at times, numerous examples are distributed throughout the cell (Figure 9.25A; Higgins and Daneo-Moore, 1974).

The papers cited above show also that not all the aerobic respiratory reactions occur within the mesosome. On the electron micrographs, poorly defined, but large, areas of the nucleoid, too, typically appear to be active in these processes. In several of the studies, no mention is made of these electron-dense regions, but Sedar and Burde (1965) state their inability to account for the dark deposits, for the regions of activity are too consistently present in the nucleoid to be explained as mere artifacts of preparation.

Unfortunately, these methods have as yet been applied to just a few bacteria, so that now it is impossible to state what body serves as the respiratory site among other major types of bacteria. Particularly needed are investigations of such forms as *Beggiatoa* and *Thiothrix* among the colorless sulfur bacteria, the Myxobacteria as a whole, and the spirochaetes. Because the mesosome appears to be a derivative of the plasmalemma, it might be anticipated that in at least some of these types the cell membrane could prove to be a site of respiration, as no mesosome is currently known to exist in any of the taxa mentioned.

Nor is it clear what the significance is of the structural differences in the mesosome found among various typical bacteria. In *Bacillus subtilis* (Van-

derwinkel and Murray, 1962) and *Chlorobium thiosulfatophilum* (Cohen-Bazire *et al.*, 1964), the membranes are arranged rather spirally and upon them are located scattered, vague, round bodies, perhaps three times the diameter of the membranes in size. Although the membranes are of trilamellar construction, frequently they seem to consist of series of smaller particles, a condition that is especially clear in the *Chlorobium* species mentioned (Figure 9.25C). On the other hand, in a second species of the same genus (*C. limicola*) described in the latter article and in *Diplococcus* (Tomasz *et al.*, 1965), the membrane becomes folded to form several smaller vesicles arranged around a larger central one. At least during the early stages of formation, the membrane here, too, is composed of series of small particles.

Furthermore, while mesosomes are frequent in gram-positive bacteria, they are often absent or poorly developed in gram-negative forms. In *E. coli* and *Proteus vulgaris* they are not present under ordinary conditions of culturing but are developed under elevated temperatures, such as 40°C. In contrast, the mesosome is as abundant in the gram-negative stalked bacteria that comprise the family Caulobacteriaceae as in the gram-positive forms (Poindexter *et al.*, 1964). Ryter and Jacob (1966) explain the difficulty in observing the mesosome in *E. coli* as resulting from the fragility of the membranes that form it; they believe that only when the plane of section falls perpendicular to the mesosomal folds are the latter visible. More recently Pontefract *et al.* (1969) confirmed these observations with refined techniques, but even with their special preparations, the organelle in micrographs was not so clear-cut as in gram-positive forms.

As will be recalled, the last named authors also hypothesized that the mesosome was essential to nuclear division, a suggestion that receives further attention in a later chapter. While interesting, their hypothesis does not receive support from a study of mesosomal behavior in *Bacillus licheniformis* (Highton, 1969). During the growth cycle of the organism, the mesosome was found to move from the end where it had been formed to the middle of the elongating cell. At this point it divided as the new cell septum grew, one product of division going to each daughter cell before cytoplasmic division was completed. Because nuclear division in exponentially growing bacteria occurs when the mesosome is still moving toward the end where division will occur, its seeming association with nuclear division is possibly more apparent than real.

9.4.2. Blue-Green Algae

The location of the actual respiratory site in the Cyanophyceae remains obscure in spite of the appearance in print of several excellent studies on the subject. In great part the present confusion results from two major factors, the first of which is the relative paucity of investigations so far conducted on the

respiration of these organisms. While this situation currently seems to be undergoing improvement, the second may prove difficult to correct, for revision of taxonomic concepts may be involved. In short, the Cyanophyceae, like the bacteria discussed in several preceding chapters, may need to be viewed as representing a number of phyletic lines, rather than a single "natural" or monophyletic group—at least ultrastructural studies on the site of respiration appear to suggest this possibility.

On one hand, Echlin (1964) reported an organelle in *Anacystis nidulans* that closely resembled the mesosome described above in the gram-negative bacteria. As in that group, the organelle consisted of tightly spiraled membranes extending into the nuclear region from the plasmalemma. However, because its actual involvement in cell respiration had not been explored, Echlin proposed that the term "lamellasome" be employed until homology of function was established. Besides possessing this similar-appearing structure, the present organism resembled the Eubacteriales in having a more or less continuous nucleoid and in lacking the polyhedral bodies and structured granules so characteristic of more typical, filamentous blue-green algae (Figures 11.1, 11.3). *Anacystis nidulans,* currently considered a unicellular type in the Chroococcales, may according to Drouet (1962) actually represent a pseudounicellular type consisting of short but multicellular filaments and should probably be assigned to the Nostocales, perhaps in the genus *Phormidium*. Similar structures have been reported from *Synechocystis aquatilis, Anabaena variabilis* (Avakyan *et al.*, 1978), and *Chlorogloea fritschii* (Baulina *et al.*, 1978).

On the other hand, filamentous members of the division possess no specific organelle of respiratory function. In an investigation of possible respiratory sites in *Nostoc sphaericum,* Bisalputra and colleagues (1969) reported that reduction of tellurite and trinito blue tetrazolium occurred upon the photosynthetic lamellae, clearly indicative of the location there of respiratory function. Similar results have been obtained in the author's laboratory (Dillon and Dillon, unpublished).

9.5. PHYLOGENETIC ORIGINS OF THE MITOCHONDRION

The problem of the phylogenetic origins of the mitochondrion has received widespread attention, based on a number of diverse observations. One proposal has suggested that blue-green algae developed the ability to engage in phagocytosis, a process claimed to be somewhat parallel to that of cell division (Cavalier-Smith, 1975). Through modification of the membranes brought into the interior by this activity, endoreticulum, lysosomes, dictyosomes, mitochondria, and the other cell organelles were thought to have developed, thereby giving rise to the eukaryotic cell. However, as no prokaryote is known to be capable of phagocytosis, the concept lacks a firm base of factual support. The same

weakness is shared by a second concept, which conceived the mitochondrion to have been derived from the peroxisome (de Duve, 1973).

9.5.1. The Endosymbiontic Concept of Mitochondrial Origins

Undoubtedly the most widely accepted concept of the origins of mitochondria, as well as those of two other organelles, has been the endosymbiontic theory. This idea was originally advanced many years ago (Wallin, 1927) but attracted little support until it was readvocated on a more sophisticated basis by Margulis (formerly Sagan) in 1967, 1968, and 1970 (Nass, 1969; Raven, 1970; Schnepf and Brown, 1971; King, 1977). Simply stated, the theory holds that the mitochondrion is a type of prokaryote that penetrated the ancestral eukaryotic cell, originally as a parasite but later acquiring mutualistic qualities. This interesting view was forwarded by the discovery that DNA was present in the organelle, thereby suggesting the existence of autonomy. At that time it was believed that the mitochondrion's entire genetic apparatus was encoded by its genome, but as shown in an earlier section, present knowledge has revealed how few of the essential enzymes, tRNAs, and ribosomal proteins are actually synthesized within that organelle.

Much additional evidence against the theory has come to light during the past decade, some of the main points being as follows:

1. The physical properties of mitochondrial ribosomes have little in common with prokaryotic ones (Table 9.1; Pace, 1973).
2. The DNA resembles that of viruses more closely than it does that of bacteria, often being circular. In many organisms, however, it is linear, thus differing even from the viral types.
3. Replication and transcription similarly involve distinctive features and are largely carried out by cytoplasmic enzymes; initiation and elongation are particularly unique.
4. The single nucleotide sequence of a mitochondrial tRNA ($tRNA^{Met}$) that has been established is neither prokaryotic nor eukaryotic.
5. Many of the elements of the respiratory cycle themselves, which supposedly enabled the invading prokaryote to become evolutionarily advantageous to the ancient host, differ strikingly from those of any known prokaryote. For example, the enzyme that catalyzes the oxidation of isocitric acid in prokaryotes requires NADP, whereas that of mitochondria utilizes only NAD.
6. The cytochromes of bacteria differ greatly from those of mitochondria, particularly cytochrome *c* and its oxidase. The cytochromes $a + a_3$ of bacteria lack the two copper atoms present in the eukaryotic enzyme and either do not oxidize mammalian cytochromes *c* or do so very slowly and the same system of mammals does not act

at all on bacterial cytochrome *c*. Consequently, any prokaryotic invader could not have aided cellular respiration as proposed.

7. Mitochondria possess an actin–myosin system that enables them to move actively about the cell, but all bacteria and blue-green algae lack such a system and comparable capabilities.
8. Mitochondria are of a number of varied types, including microvillose and septate, not to mention the numerous configurations found in chloride cells and other specializations described in the preceding pages. Different types often occur in the varied tissues of the same organism and several distinct populations may occur together. No prokaryote is extant that can assume so many diverse ultrastructural traits.
9. Mitochondria give rise to highly specialized cell parts such as the kinetoplast of hemoflagellates, the spiral at the base of the metazoan sperm tail, and the inner segment of vertebrate retinal cells, adaptations impossible to reconcile with their being a prokaryotic invader.
10. These organelles often actively participate in such cell activities as mitotic and meiotic division and the condensation of chromatin in sperm nuclei, functions much more amenable to the organelle's being a specialized adaptable part of a cell rather than an endosymbiont.
11. In yeasts, the organelle can arise from open sheets of membranes, and in other cells *de novo* origins have not been completely ruled out—observations not at all compatible with the endosymbiontic concept. Consequently, it appears no longer possible to consider this organelle other than an evolutionarily derived, specialized compartment of the evolving eukaryotic cell itself.

9.5.2. The Episome Theory

Dissatisfaction with the weaknesses of the endosymbiont concept stimulated the proposal of other views (Cohen, 1970), the most outstanding of which was the episome theory (Raff and Mahler, 1972, 1973). Episomes are plasmids that are attached to the nucleoid of bacteria, plasmids being covalently closed-circular DNA molecules that replicate independently of the nucleoid DNA. Hence, the nucleoid of the mitochondrion may be loosely viewed as a eukaryotic plasmid. In brief, the hypothesis suggests that the organelle developed from the ancestral eukaryotic plasmalemma, combining with an episome (plasmid) that carried the genes for mitochondrial ribosomes, tRNAs, and some of the respiratory enzyme subunits. Unfortunately, the data employed in support of the proposed derivation are drawn mainly from the ribosomes and genome of the mitochondrion, a source already shown to be too controversial to provide substance to phylogenetic considerations (Uzzell and Spolsky, 1973). The same statement holds true also for a closely related proposal (Reijnders, 1975), as

well as for the analyses of sequence differences of various nucleic acids and proteins presented by Schwartz and Dayhoff (1978). The composite evolutionary tree of the latter clearly indicates the unreliability of difference counts, as mammals come off the tree at two widely separated points and "plants" (metaphytans) at three. A related concept has postulated that a part of a prokaryotic nucleoid was transferred into a eukaryotic cell by a virus (Ostroumov, 1977).

9.5.3. A Biological Concept

Actually the episome concept agrees well with many factual observations of which its authors appear to have been unaware; in short, its main flaws are a lack of sufficient depth of supportive data and a restricted view of the nature of mtDNA. However, the genome of the mitochondrion does not necessarily represent a prokaryotic episome or plasmid; its frequent linearity demonstrates that point clearly. In trying to decipher the probable past events in the development of this organelle, some facts disclosed by the analyses of the respiratory cycle, the cytochromes, and ribosomal 5 S RNA need to be recalled. These largely pertain to the existence of several evolutionary levels among the prokaryotes over and above those of bacteria and blue-green algae. Rather, the colorless sulfur bacteria *(Beggiatoa, Thioploca,* and related genera) need to be considered a separate phylogenetic stage, apparently at the simplest known cellular level above the rickettsiae; *Clostridium* (at least in part) is shown to be somewhat more advanced. Then there exist two, or perhaps more, successively higher levels among the blue-green algae, followed by a series of stages among the bacteria proper. Thus *E. coli* and *Bacillus megaterium* do not represent the bacteria as a whole; they can be considered only as representatives of separate lines from among the numerous ones that exist within that group. That the true yeasts are the most primitive of the existing eukaryotes has previously been indicated here by several sets of evidence. The euglenoids then come off the main line at some distance above the yeasts, followed by a series of steps leading to the remainder of the protistans, and finally to the branches ending in the metaphytans and metazoans.

Accordingly a logical sequence of events in mitochondrial phylogeny could be as follows:

1. Because the earliest functional respiratory cycle occurs in the blue-green algae in which it is located on the photosynthetic thylakoids, themselves derivations of the plasmalemma, the respiratory functions might be considered to have been situated originally on the plasmalemma. Further discussion of the relations that exist between respiration and photosynthesis is provided in the chapter on the chloroplast that follows.

2. In the second (higher) branch of blue-green algae, these two sets of processes became separated as the mesosome formed. This organelle also is a derivative of the plasmalemma; that is, it is derived from a particular region of the plasma membrane that is specialized for the tricarboxylic acid cycle. Thus the groundwork was set for the eventual formation of the two separate specialized organelles, the mitochondrion and chloroplast.
3. Through the several lines of eubacteria that followed, cell respiration and photosynthesis essentially remained properties of separate regions of the plasmalemma; mesosomes typically were present, but in cases where they have failed to differentiate from that membrane, no serious problem is presented—the ability to produce intracellular membranes specialized for respiratory functions has still persisted.
4. In ancestral eukaryotes, these intracellular membranes seem to have undergone radical modification, as intimated by those present in oxygen-deficient cultures of yeast. Such organisms have the membranes present as compressed vesicles, each consisting of double membranes as shown in Figure 9.13A.
5. The membranes later became increasingly folded over on themselves, thereby gradually enclosing a matrix. At first, these folded membranes remained slightly open. It is during this stage, represented among yeast transferred into an oxygen-rich environment, that a genome was taken in along with the ground substance. The source of the DNA could have been a plasmid (episome), as Raff and Mahler (1972) have proposed, but the frequent occurrence of linear molecules militates against that concept. Its actual origin could readily be investigated through the use of yeast recovering from anaerobic conditions as the experimental subject. At the same time, ribosomes also were taken into the enclosed matrix, probably then being identical to those of the cytoplasm. As with the DNA, it should also be possible to investigate the actual origins of these particles in the anaerobic yeast system.
6. At the level of aerobically grown yeast, this premitochondrion closed entirely and developed cristae, to become a complete but primitive mitochondrion.
7. Following its origin in this manner, the organelle underwent a series of changes, as indicated by the diagram. Among the alterations not shown seems to have been a gradual loss of much of its genome, especially in the line leading to the metazoans. Such losses appear to have occurred independently along several side branches, accounting for the inconsistencies in the encoded tRNAs that have been demonstrated among the various major taxa.
8. It should be especially noted that the acquisition of a genome and ribosomes occurred in a stock prior to the earliest actually known

eukaryote. As this stock would have been evolutionarily between the most advanced prokaryote and the lowest living eukaryote, the likenesses to and dissimilarities from the DNA and ribosomes of both types of organisms are accounted for.