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# Mitochondrial DNA: Advances, Problems, and Goals

Studies of size and structure of mitochondrial DNA relate to biogenesis and function of this organelle.

Margit M. K. Nass

The concerted efforts of biologists and biochemists have led to the discovery that deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are distinct components of cytoplasmic organelles, in particular of mitochondria and chloroplasts. Various problems pertaining to the heredity and biogenesis of mitochondria and chloroplasts, and to the structure, function, and synthesis of nucleic acids and proteins in these organelles, have been reviewed (*1–8*). It is characteristic of our present age of rapid scientific developments, involving exponentially growing numbers of individuals and teams, that the pertinent literature on mitochondrial DNA, which was in its infancy in the early 1960's (*8*), far exceeds the scope of an article in these pages today. I restrict this discussion, therefore, to the more recent advances in the structure and biochemistry of mitochondrial DNA and present some of the problems we confront at the present level of knowledge and insight (*9*).

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## Structure and Size of DNA Molecules

The most common conformation of mitochondrial DNA of multicellular animal cells is a double-stranded circle with a perimeter of 4.7 to 5.5 microns (*10, 11*) corresponding to a molecular weight of $9 \times 10^6$ to $10 \times 10^6$ daltons. Circular mitochondrial DNA has been described in most classes of vertebrates, including man (*12–15*), birds (*16*), and amphibians (*17*); it was found in sea urchin (*18*) and as a minor DNA component of mitochondria in yeast (*19*). The circular DNA molecules observed in the electron microscope consist of a mixture of highly twisted forms and loosely twisted, or open, types (Fig. 1). The twisted structure is typical of covalently closed DNA, and the loosely twisted forms may represent DNA with one or more single-strand scissions (nicked DNA). Other factors, however, are known that determine the degree of coiling. The DNA molecules can be separated on gradients consisting of cesium chloride and ethidium bromide by use of the principle that less dye (at high concentrations) binds to covalently closed circles than to nicked or linear DNA, and different buoyant densities are imparted to the various molecules regardless of base composition (*12*).

The very small differences in length that have been observed for mitochondrial DNA's from various cell types are frequently due to technical factors in the hands of different investigators. Generally, the ionic strength of the medium (hypophase) upon which the DNA molecules are spread as a DNA-protein monolayer affects the molecular lengths significantly (*20*). The molecules may shorten by about 10 percent at ionic strengths above 0.1 mole per liter, compared with distilled water as a hypophase. The existence of true size differences was shown by spreading different mitochondrial DNA's together as mixtures. The measurements of size obtained from a mixture of mitochondrial DNA from mouse fibroblasts (L cells) and from chicken liver followed a bimodal distribution with peak categories (4.7 to 4.8 $\mu$ and 5.1 to 5.2 $\mu$, respectively) corresponding to the size observed when each DNA was spread individually. The existence of small size

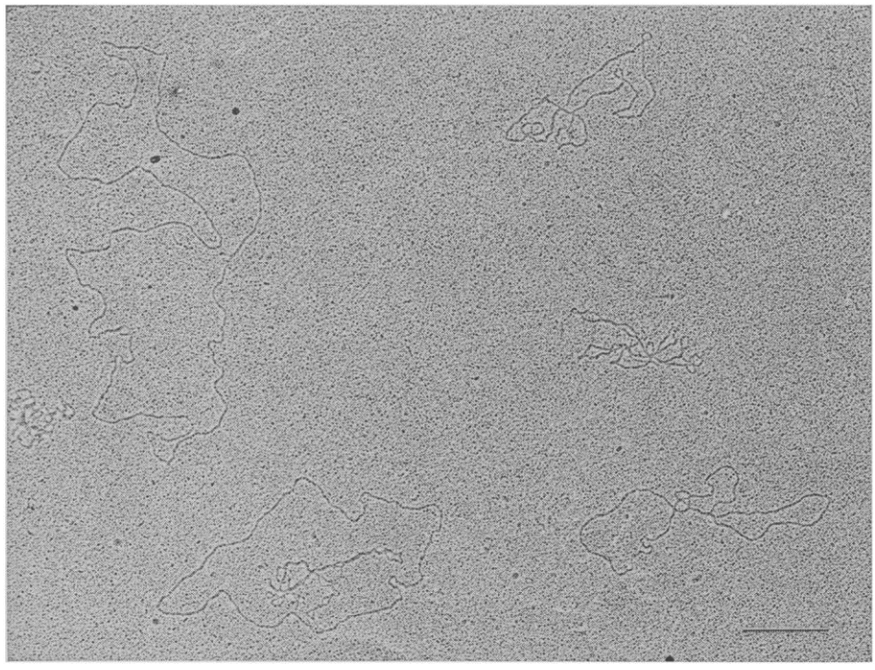

Fig. 1. Electron micrographs of circular DNA molecules isolated from mitochondria of mouse fibroblasts (L cells). A highly twisted or supercoiled monomer is lying inside an open monomer (lower left); three loosely twisted monomers (right); one open dimer (upper left). The molecules have been spread on the surface of a monolayer of protein and subsequently contrasted by shadow casting from all directions with vaporized platinum-iridium. Scale is 0.5 $\mu$.

differences was similarly confirmed for mitochondrial DNA of ascites tumor cells and human liver (*15*).

Another group of DNA molecules that are twice or several times the size of 5 $\mu$ has been described in mitochondria derived from several mammalian cell types (*2*, *12–15*) and from sea urchin eggs (*18*). These molecules are open circular dimers (Fig. 1) or dimers and oligomers consisting of interlocked units (Fig. 2). Interlocking of molecules can usually be distinguished from mere overlapping (which occurs rarely) by focusing of the electron microscope and by enhancing the three-dimensional image of the structures by successive cycles of rotary and undirectional shadow casting with vaporized metal. In cesium chloride–ethidium bromide gradients, interlocked circles are found in a band of intermediate density between the lower region containing covalently linked monomers and dimers and the upper band containing nicked circles and linear DNA. It has been suggested that the interlocked dimers are found in the middle band because one member is nicked and the other is covalently linked (*13*). The fact that the dimers have a stable covalent bond is evidence that they are formed in vivo rather than that they arise as artifacts during isolation. Moreover, in our laboratory, dimers have been released directly from isolated L-cell mitochondria by osmotic shock, indicating that dimers occur in intact mitochondria (*15*). Multiple forms of DNA have also been reported in viral DNA and other DNA's of microbial origin (*21*). The functional significance of multiple forms of DNA is not yet understood. They may represent products of DNA replication or recombination or both.

The circular structure of mitochondrial DNA does not appear to be universal. The bulk of yeast mitochondrial DNA has been reported to consist of linear filaments (*19*, *22*), 4.5 to 5 $\mu$ in length, some of which have "sticky ends" or protruding single-stranded chains that can be annealed to form circles (*19*). Lambda bacteriophage DNA isolated directly from the virus has a similar structure and can similarly be made to circularize. Some linear dimers, oligomers, and covalently closed circles (2 to 3 percent) were also found in the DNA of yeast mitochondria (*19*). Undoubtedly, the structure and size of yeast mitochondrial DNA is not yet clarified. The problem of degradation of DNA by shear or by hydrolytic enzymes may be considerably more serious in this cell type than in cells of higher organisms. A deficiency in the polynucleotide-joining enzyme ligase may also be responsible for the presence of linear or nicked DNA. It is possible, however, that the structure of yeast mitochondrial DNA represents a specialized case, distinct from the structure of circular DNA of higher forms and possibly also distinct from the mitochondrial DNA of many other eukaryotic microbial and plant cells. The latter DNA's appear to be linear also but are reported to have a higher molecular weight than that corresponding to the 5-$\mu$ circles (*23–25*).

## DNA Content in Individual Mitochondrion

The amount of DNA present in individual mitochondria is of particular interest because it provides a basis for estimates of the potential informational content or coding capacity of mitochondrial DNA. All existing evidence suggests that the total DNA content per mitochondrion from different cell types is variable and corresponds to at least one or two molecules whose molecular weight is characteristic to each cell type. The combined DNA content of all mitochondria per cell is very small compared with the amount of DNA found in the nucleus. For example, a mouse fibroblast cell grown in culture (L cell) contains about 250 mitochondria; yet the combined DNA of the mitochondrial population corresponds to only 0.15 percent of the DNA located in the cell nucleus.

Essentially three experimental approaches have been used to study DNA content of mitochondria: (i) electron microscopic examination of DNA in ultrathin sections of mitochondria (*6*, *26–28*); (ii) chemical analyses of DNA extracted from mitochondria by hydrolytic procedures or isolated on the basis of specific buoyant density in cesium chloride gradients (Table 1); (iii) isolation of mitochondrial DNA in cesium chloride–ethidium bromide gradients, which separate DNA molecules on the basis of their molecular topology (Table 2).

Several general conclusions can be drawn from these analyses. (i) The DNA content per milligram of mitochondrial protein from highly differentiated, slow-growing tissues is smaller than that of fast-growing cells, for example, embryonic tissues and cells grown in culture. The highest DNA

content of mitochondria occurs in tumor cells. It is possible that hormones (*28*) also cause increases of DNA filaments in mitochondria. (ii) The DNA content of unicellular microorganisms and plant cells is similar to that of slow-growing animal cells if related to protein, but higher if expressed per organelle. (iii) The lowest DNA content of mitochondria reported corresponds to two molecules of DNA of molecular weight 9 to 10 $\times$ $10^6$ per organelle. Up to six molecules is common (for example, L cells). (iv) Multiple-length molecules (dimers, interlocked dimers, and oligomers) have been found in mitochondria of mammalian cells, especially rapidly growing and malignant cells, and in echinoderms. The data are still insufficient to allow speculations regarding the relative occurrence and significance of various molecular forms in normal and malignant cells. Nevertheless, it is tempting to try to correlate the suggestive evidence that tumor cells contain fewer mitochondria per cell but more interlocked DNA than normal cells. The inability of multiple DNA molecules to separate (regardless of the mechanism of their formation) may stop mitochondrial division, or, vice versa, arrest of mitochondrial division may lead to the accumulation of unseparated DNA molecules.

There are several sources of error that have not yet been sufficiently controlled in many of the examples tabulated in Tables 1 and 2. (i) One problem is contamination of mitochondria by adsorbed nuclear DNA. This error may be reduced or eliminated by digestion with deoxyribonuclease, which generally does not penetrate intact mitochondria, or by selecting mitochondrial DNA from cell types where nuclear and mitochondrial DNA have different buoyant densities. (ii) The value of DNA expressed per milligram of mitochondrial protein is subject to error because the protein content per mitochondrion varies in different cell types or even in a population of mitochondria from the same tissue. Mitochondria are highly pleomorphic, often branched, sometimes of giant size, and they may differ in the relative proportion of internal membranes (cristae) and remaining matrix; for example, mitochondria from fast-growing and tumor cells tend to have fewer cristae and more "open spaces" in their matrices than organelles from functionally differentiated and active tissues, such as liver and muscle. The former mitochondria could contain less protein than the latter, which would be reflected in higher DNA values. As with L cells, most values of DNA content should thus be verified by relating it to actual counts of mitochondria performed on the same samples, provided no nonmitochondrial particles contaminate the preparations and are mistaken for mitochondria in counts. (iii) The degree of DNA extraction may vary not only with different methods employed but also with the functional state of the DNA. For example, a minor but functionally distinct proportion of mitochondrial DNA (conceivably DNA in the process of replication or transcription) may resist, to some degree, available methods of isolation. This question has not yet been adequately tested in different types of mitochondria (*29*). Never-

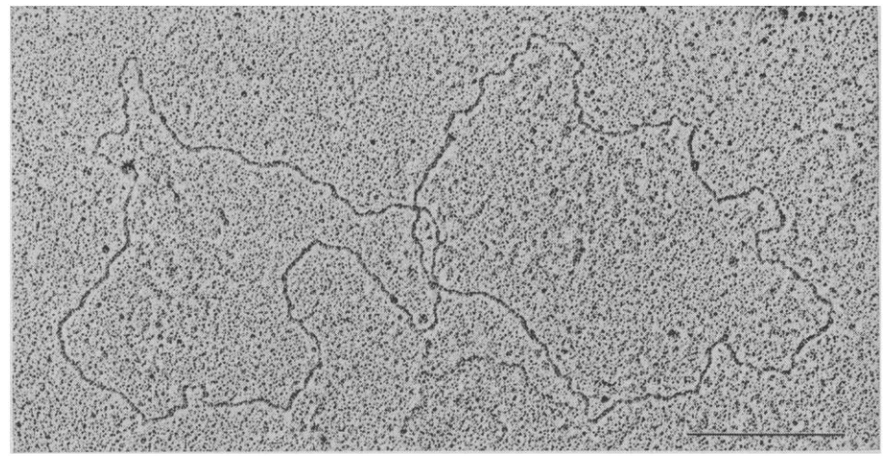

Fig. 2. Electron micrograph of a circular DNA dimer consisting of two interlocked monomers, isolated from mitochondria of mouse fibroblasts (L cells) in the intermediate band of cesium chloride–ethidium bromide gradients. Scale is 0.5 $\mu$.

Table 1. The content of DNA and protein in mitochondria.

| Cell type | DNA related to mitochondrial protein ($\mu$g/mg) | Protein per single mitochondrion (g $\times$ $10^{-13}$) | DNA per single mitochondrion (g $\times$ $10^{-16}$)* |
|---|---|---|---|
| | *Adult tissues* | | |
| Beef heart (*75*) | 0.24 | 1.1 (*76*) | 0.26 |
| Rat liver (*45*, *46*, *75*, *77*) | 0.65, 0.8<br>0.46, 0.51 | 2.0 (*78*) | 0.9–1.6 |
| Hamster liver (*77*) | 0.58 | | |
| Mouse liver (*77*) | 0.55 | | |
| Chicken liver (*16*) | 0.5 | | 0.7 |
| | *Eggs (nondividing)* | | |
| Frog oocytes (*79*) | 0.52 | | |
| Sea urchin (*80*) | | | 0.23 |
| | *Rapidly growing cells* | | |
| L cells (mouse fibroblasts) (*16*) | 1.1, 1.0–1.2† | 0.80 | 0.88 |
| Mouse embryo (*77*) | 1.3 | | |
| Mouse placenta (*77*) | 0.9 | | |
| Rat embryo (*77*) | 1.8 | | |
| Rat placenta (*77*) | 1.7 | | |
| Hamster embryo (*77*) | 1.3 | | |
| Hamster placenta (*77*) | 1.1 | | |
| | *Tumors* | | |
| Ascites tumor (mouse) (*24*) | 2.5 | | |
| Hepatoma (rat) (*77*) | 5.3 | | |
| Walker-carcinoma (rat) (*46*, *77*) | 4.7, 8.2 | | |
| Jensen-sarcoma (rat) (*46*, *77*) | 4.7, 5.4 | | |
| Sarcoma (mouse, drug-induced) (*77*) | 4.7 | | |
| Sarcoma (hamster, polyoma virus–induced) (*77*) | 4.7 | | |
| | *Eukaryotic microorganisms* | | |
| Yeast (*58*, *81*) | 0.7–1.0, 1.1–4.3 | 0.85 (*7*) | 0.6–3.7 |
| *Tetrahymena* (*82*)† | | | 3.7 |
| *Neurospora* (*25*) | 0.7 | | |
| | *Higher plants* | | |
| Mung bean (*83*)† | 0.8 | 6.2 | 5.0 |
| Turnip (*83*)† | | | 5.0 |

* A molecular weight of 60 $\times$ $10^6$ daltons corresponds to 1.0 $\times$ $10^{-16}$ g of DNA. † Mitochondrial DNA was isolated on cesium chloride gradients rather than extracted by acid hydrolysis.

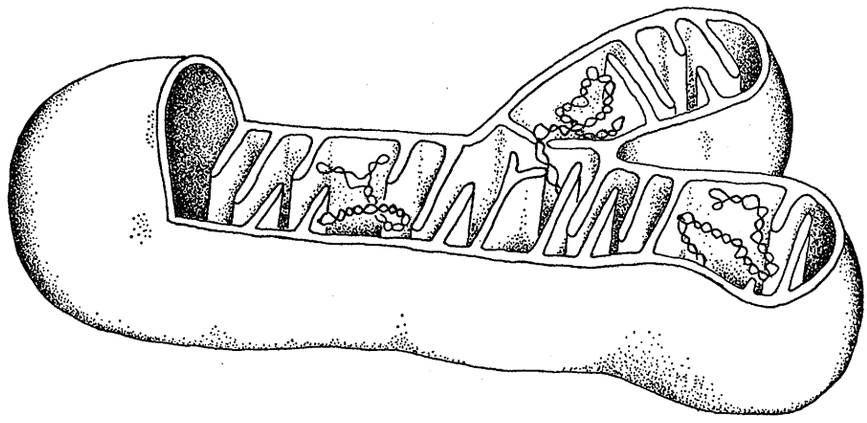

Fig. 3. Three-dimensional representation of the possible arrangement of circular DNA within a mitochondrion. A branched mitochondrion typical of L cells and many other cell types emphasizes the polymorphous structure of these organelles. The number of nucleoids containing DNA molecules is variable. The DNA molecules may be attached to portions of the membranes. The DNA circles (half-length 2 to 2.5 $\mu$) may be coiled or at least folded inside the matrix compartments which are about 0.5 $\mu$ in diameter. The twists or supercoils shown on the DNA do not necessarily reflect their occurrence or number in vivo.

theless, despite the great diversity of cell types, of investigators, and of preparative or analytical methods used, the average DNA content per mitochondrion is within a remarkably small range.

It will now be necessary to distinguish between two possible conditions that would lead to an elevated concentration of DNA per organelle: (i) net increases of DNA that parallel the amounts of protein during growth of the organelle (prior to division) and (ii) net increases of DNA per organelle that exceed the net amounts of proteins made, regardless of whether net protein synthesis is normal or deficient.

What would be the reason for increased concentrations of DNA in mitochondria? Experiments with dividing bacteria indicate that increases in total DNA content are associated with higher growth rates and are achieved by having more than one site of DNA replication, leading to cells with two or more nucleoids (*30*, *31*). In fast-growing bacteria, in contrast to slow-growing forms, a new round of DNA replication may begin before the previous one has ended, leading to multiple replication forks (*30*). If a similar process occurred in mitochondria this would be difficult to detect because the DNA molecules are about 200 times shorter than the DNA molecules of bacteria. Perhaps the clusters of circular molecules that are found joined to each other at a common point after release by osmotic shock from mitochondria of dividing L cells (*15*) are products of multiple replication. It is also possible that mitochondrial DNA in fast-growing cells does not turn over (or become degraded) as rapidly as in slow-growing cells and has therefore an increased stability during rapid growth, as suggested by studies on DNA turnover in mitochondria (*32*). At any rate, the task ahead is further complicated by the distinct possibility that functional heterogeneity of a mitochondrial population may exist within one cell and among cells of the same tissue.

## Intramitochondrial Arrangement of DNA

How are DNA molecules structurally arranged within mitochondria? From various data outlined in this and the following two sections, a three-dimensional model of a mitochondrion has been derived (Fig. 3). Its main purpose is to serve as a visual aid to readers rather than to claim that the problem of DNA arrangement in mitochondria has been solved. It will be evident from the discussion below that we are still far from such a position.

Studies with electron microscopy have shown that mitochondria from cell types representing the major animal phyla and some plants contain at least one area in their matrix in which DNA is located (*6*). Examination of serial sections of mitochondria indicated that the extensively branched organelles from chick embryo muscle contain up to six DNA-containing areas or "nucleoids," at least one in each branch. Serial sections of the filamentous mitochondria of L cells also showed variable numbers of nucleoids, mostly two to four areas and sometimes up to six (*15*). The DNA regions are frequently separated into compartments by cristae; these discrete areas are especially well seen in some tumor cells. It is not yet known whether an interrelationship exists between these nucleoids. Mitochondria may thus contain multiple nucleoids like bacteria.

Does each DNA region in a mitochondrion consist of one or more DNA molecules? This question is very difficult to answer for technical reasons. In our laboratory the problem was experimentally approached by analyzing, in the electron microscope, displays of DNA released by osmotic shock from isolated individual mitochondria of L cells (*11*, *15*). The mitochondria must be highly diluted and carefully dispersed to avoid overlapping. The procedure is carried out on a monolayer film of protein upon which the fragmented mitochondria and liberated DNA molecules adsorb (Fig. 4).

Essentially there were two types of findings. (i) A collection of two to six circular molecules was found with remnants of a mitochondrion. This amount has been assumed to represent the total isolatable DNA content of a single organelle, in view of the distance separating interfering mitochondria and the agreement with values obtained by chemical analyses. (ii) Frequently, two circular monomers were joined at one point, or two circular monomers and one dimer were joined at a common knob-like point (*15*). It is possible that the joined molecules represent the content of one nucleoid. Generally it appears that the content of one nucleoid may vary, containing mostly one or two monomers and sometimes mixtures of monomers and dimers. The difficulty of interpreting molecules from closely adjacent nucleoids of a mitochondrion, however, is obvious. Another factor of some uncertainty is the degree of lysis of a mitochondrion. For this reason, therefore, experiments of this type were supplemented by other analyses (for example, serial sections of nucleoids and chemical analyses related to individual mitochondria). More definitive answers may have to await a hypothetical, technologically more advanced age when mitochondria may be microdissected at magnifications of the electron microscope.

## Membrane-Association of DNA

Evidence from electron microscopy suggests that DNA molecules, perhaps during certain physiological states, are not freely suspended in the mitochondrial matrix. In many cell types, DNA filaments can be seen attached to the mitochondrial membrane system, either in ultrathin sections of mitochondria or in preparations of DNA liberated by osmotic shock from isolated intact mitochondria (*5*, *6*, *15*). In sections, membrane attachments are apparent not only in mitochondria known to have circular DNA but also in mitochondria of protozoans (*6*) that probably have linear DNA. In the osmotic shock experiments, membrane association of DNA occurred with highest frequency in mitochondria from rapidly growing cells (L cells and ascites tumor cells); mitochondrial DNA of adult tissues like rat and chicken liver had few such connections (*15*). It is not yet established whether these findings may reflect a functional significance of the attachments or merely a preparatory artifact. In favor of the former possibility, DNA added to mitochondria before spreading did not artificially adsorb to membrane fragments. The nature of these DNA-membrane attachments remains to be elucidated. Preliminary evidence has shown some dissociation in response to treatments with the enzyme mixture pronase, but not with ribonuclease or phospholipases (*15*). Ultrastructural studies of bacteria have also shown connections of DNA fibrils with the bacterial membrane at the site of the mesosome (*33*) where many respiratory components are located. The connection of DNA with the membranes has functional implications in DNA replication and transcription. In mitochondria, as in bacteria, however, the occurrence and definition of these connections will require extensive additional studies.

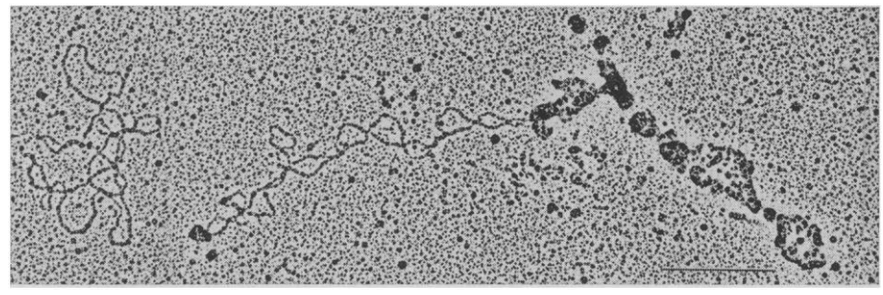

Fig. 4. Electron micrograph of circular DNA molecules released from isolated mitochondria by osmotic shock. A loosely coiled molecule is seen associated with a ruptured mitochondrion (right). One molecule is lying free (left). Scale is 0.5 $\mu$

## Is Mitochondrial DNA Supercoiled in vivo?

Whether twists or supercoils occur in covalently closed circular DNA molecules synthesized in vivo has been widely discussed but not yet solved. The presence or absence of supercoils in vivo is of obvious significance in the understanding both of the structural organization of circular DNA molecules inside mitochondria (and other cell organelles or viruses) and of the mechanism of DNA synthesis, replication, and transcription.

Physical studies of closed circular DNA of viruses (*34*, *35*) have shown that a covalently closed, double-stranded DNA molecule may convert into a right-handed twisted (supercoiled) structure if additional turns have been wound into the Watson-Crick helix (which may be underwound at the time of ring closure in vivo). A one-to-one correspondence exists between the winding or unwinding of one complete turn of the Watson-Crick helix and the winding or unwinding of a superhelical turn. This relation is a consequence of the invariance of the winding number in covalently closed molecules where the DNA strands are topologically locked and cannot rotate freely. These observations were derived from studies involving titration of closed circular DNA with intercalating dyes or hydroxyl ions that cause unwinding of the double helix and quantitatively equivalent unwinding of the supercoils. Essentially two alternatives have been suggested for the origin of supercoils. (i) The DNA molecule is wound around a core substance at the time of ring closure, and (ii) the DNA molecule is partially unwound at the time of closure—that is, it has a greater pitch or fewer Watson-Crick helical turns per nucleotide at final closure in vivo than during purification in vitro (for reasons still unknown).

There appears to be a proportionality of supercoiling density and molecular

Table 2. Chemical and electron microscopical estimate of circular and linear DNA molecules isolated from mitochondria in three bands on cesium chloride–ethidium bromide gradients (DNA I, lower band; DNA Im, intermediate band; DNA II, upper band).

| Mitochondria | | DNA I Circular monomers, dimers (covalently closed) | | DNA Im Circular monomers, dimers interlocked dimers, oligomers | | DNA II Nicked circles, some oligomers, linear DNA | |
|---|---|---|---|---|---|---|---|
| Source | Deoxy-ribo-nuclease* | DNA/ protein ($\mu$g/mg) | Monomers : dimers (%) | DNA/ protein ($\mu$g/mg) | Monomers : dimers (%) | DNA/ protein ($\mu$g/mg) | Circles : linear (%) |
| L cells (*15*) | — | 0.44 | 94 : 6† | 0.08 | 24 : 76‡ | 3.9 | 1 : 100 |
| L cells (*15*) | + | 0.56 | 95 : 5 (48% of total) | .12 | 21 : 79‡ (10% of total) | 0.50 | 70 : 30 (42% of total) |
| HeLa cells (*15*) | — | | 90 : 10§ | | 40 : 60 | | |
| Normal leukocytes (*14*) | — | | 99 : 1 | | | | |
| Leukemic leukocytes, Case 1 (*14*) | — | | 68 : 32‡ (15 to 50% of total) | | | | |
| Case 2 (*14*) | — | | 89 : 11‡ | | | | |
| Sea urchin whole eggs (*18*) | — | | 95 : 5 | | 33 : 67 | | |

* If treatment of mitochondria with deoxyribonuclease is omitted, variable amounts of nuclear DNA are present as linear DNA. † 80 to 90 percent of dimers consist of open double-size circles; the others, of two joined monomers. ‡ Figures include interlocked dimers and 1 to 3 percent interlocked oligomers. § Dimers consist of two joined monomers.

weight, corresponding to about 3.7 tertiary turns per $1 \times 10^6$ molecular weight. The average number of supercoils per molecule in purified DNA is 12 to 15 for polyoma and SV40 virus DNA (*35*), 18 for papilloma virus DNA (*36*), and 33 to 40 for mitochondrial DNA of L cells (*15*) and of chicken liver (*15*, *37*). The respective molecular weights are $3.3 \times 10^6$, $4.9 \times 10^6$, and $9 \times 10^6$ to $10 \times 10^6$ daltons. The number of crossovers of DNA strands seen in electron micrographs corresponds closely to the number of supercoils determined by dye-binding and titration studies.

It is unfortunate that DNA molecules cannot be viewed in their native state in vivo. The possibility that DNA molecules in vivo are not supercoiled at all must also be considered. For example, the thermal and ionic environment existing at the time of ring closure of viral DNA synthesized in vitro seems to determine the degree of supercoiling (*38*). Uncoiled molecules could be synthesized, and supercoils could be introduced and removed experimentally. The conditions prevailing in vivo within the mitochondrion or in other parts of the cell where viruses are synthesized obviously are unknown, and therefore cannot be duplicated in vitro so that we can ascertain whether supercoils exist under these conditions. To avoid some of the influences that affect DNA structure during purification, we have examined the coiling of DNA liberated by osmotic shock from intact mitochondria because the molecules are fixed instantly after release on a protein film. The circular DNA released from mitochondria of rapidly growing L cells and ascites tumor cells was found to be mostly open or loosely coiled (Fig. 4), with relatively few highly twisted circles present. In contrast, DNA liberated from mitochondria of livers from adult rats and young chickens was mostly tightly coiled (with 25 to 35 twists per molecule) (*15*). If all molecules were originally supercoiled in vivo, the open molecules in fast-growing cells may be explained by the preferential liberation of an endonuclease which introduces single-strand nicks in the DNA during osmotic shock and thereby causes unraveled molecules. Alternatively, many circular molecules may be open as a result of DNA replication or other functions which are not as active in mitochondria of resting or slow-growing cells.

## Physicochemical Properties of Mitochondrial DNA

The physicochemical properties of the circular forms of mitochondrial DNA have been studied extensively and found to conform with most criteria that have been established to describe the properties of intact and nicked circular DNA of polyoma and papilloma viruses (*34*). To summarize, the twisted supercoiled circular DNA, which predominates in fresh preparations of circular viral or mitochondrial DNA, is covalently closed (component I), and the open or loosely twisted circular form has one or more single-strand scissions (component II). The former can be converted to the latter by the introduction of single-strand breaks, which allows the molecule to rotate around the bond opposite the break and thus release the supercoils. The properties of component I differ from those of II because of the topological restraint to unwinding of the double-stranded helix, imposed by the covalently closed structure. Consequently, component I, as compared to II or linear DNA, greatly resists denaturation or strand separation by heat and alkali; it renatures more easily after denaturation because the two strands remain aligned and "zip" together as hydrogen bonds reform. The hydrodynamically more compact component I also has a higher sedimentation velocity at neutral *p*H and, more so, at alkaline *p*H; it also has a higher buoyant density in alkaline cesium chloride. Furthermore, component I binds less of the intercalating dye ethidium bromide at high concentrations of dye than nicked circular and linear DNA does, and therefore bands at higher density in cesium chloride–ethidium bromide gradients. The details underlying these properties have been discussed for viral DNA (*34*, *35*). The properties of covalently closed mitochondrial DNA are summarized in Table 3.

The structure of mitochondrial DNA of yeast has been particularly controversial, and Shapiro *et al.* (*19*) have reported that the bulk of this DNA consists of linear 4.5-$\mu$ filaments some of which possess cohesive termini which can be annealed to form hydrogen-bonded (but not covalently closed) rings. In our laboratory, we have examined the open circular and linear forms of DNA component II found in highly purified L-cell mitochondria (30 to 40 percent of the total mitochondrial DNA) for similar distinct properties (*15*). However, cohesive ends could not be detected in this mammalian DNA component II. In these tests, nicked circles did not form linear molecules upon heating followed by quick cooling in ice, and linear molecules could not be annealed to form circles. It may be assumed that these forms of DNA II in L cells arose from closed circles that were nicked on one or both strands at different and opposite sites, and that the nicks are either due to damage during isolation or to some biologically significant function. In models of DNA replication in bacteria (*31*), it has been postulated that one of the two DNA strands must be broken at one time for replication to proceed.

## Base Composition

The average base compositions of nuclear DNA's of closely related organisms tend to be very similar. In view of the strikingly similar size of mitochondrial DNA from diverse cell types, a possible common evolutionary origin of mitochondria might be expected to be reflected in similarities of DNA base composition. Expressed as buoyant densities (which relate to the guanine plus cytosine content of DNA), mitochondrial DNA's of many organisms tend to have similar average base compositions (mammals, 1.698 to 1.704 g $cm^{-3}$; chicken, 1.707 g $cm^{-3}$; frog, 1.702 g $cm^{-3}$; sea urchin, 1.704 g $cm^{-3}$; and higher plants, 1.706 g $cm^{-3}$). Nevertheless, diverse values have been reported, especially for unicellular microorganisms (*Euglena,* 1.691 g $cm^{-3}$; yeast, 1.682 g $cm^{-3}$; *Tetrahymena,* 1.682 g $cm^{-3}$; and paramecium, 1.702 g $cm^{-3}$) (*2*, *4*). Mitochondrial DNA of some species of *Neurospora* have DNA populations banding at different densities (*39*). The buoyant densities of mitochondrial DNA may be identical, similar, higher, or lower than those of nuclear DNA of the same cell type, with no clear relation to phylogenetic position of the organism. It is clear that a phylogenetic relation of mitochondrial DNA cannot be evaluated from analyses of mean base composition. On the other hand, it is of interest to note that the DNA of bacteria is known to be highly mutable and that the range of base composition is wider than the corresponding range for the DNA of nucleated cells. Evidence that mitochondrial DNA can mutate has been found in certain cyto-

plasmic yeast mutants that contained mitochondrial DNA with altered base composition.

Clearly, the base sequences rather than the average base composition must be investigated in mitochondrial and nuclear DNA. Qualitative hybridization studies involving combined density-gradient centrifugation and electron microscopy of the products suggested that base homologies exist between mitochondrial DNA of two vertebrates (salamander and chicken) but not between mitochondrial DNA of salamander cells and yeast (*40*). However, well-controlled quantitative hybridization will be required to further analyze this problem. A reaction of mitochondrial DNA with nuclear DNA is not detectable in hybridization studies at the present level of sensitivity. Nuclear DNA molecules of most organisms are several hundred times longer than mitochondrial DNA molecules, and even if a segment of base sequences is homologous with a segment on mitochondrial DNA, it would be difficult to detect. The nearest-neighbor frequencies of dinucleotides ending with guanine have been studied in mitochondrial and nuclear DNA from slime molds by analyses of RNA copies synthesized in vitro on templates of each DNA (*41*). It was concluded that the doublet frequencies of mitochondrial DNA were closer to the random frequencies typical of prokaryotic microorganisms than those of nuclear DNA. However, evidence that both DNA strands had been copied, as well as analyses of highly purified mitochondria from other organisms, is required to allow generalizations.

The population of DNA molecules from mitochondria of the same cell type appears to be highly homogeneous, as judged from renaturation kinetics of mitochondrial DNA (*16*). In our laboratory, we are now analyzing the homogeneity of mitochondrial DNA molecules within the cell and among different organisms (*2*). The analyses involve mapping by electron microscopy of specific denaturation sites rich in adenine and thymine on molecules derived from mitochondria of the same cell type and from other sources (*2*). Denaturation is induced by heating with formaldehyde. Viral DNA from lambda bacteriophage and polyoma virus has been similarly studied (*42*), and sites of denaturation occurred at reproducible locations on all molecules from each virus.

Table 3. Physical properties of covalently closed mitochondrial DNA. The data are tabulated according to the description of closed circular viral DNA (*34*).

| Properties | Source of mitochondrial DNA | | | | |
|---|---|---|---|---|---|
| | L cells (mouse) | Leukocytes (human) | Liver (chicken) | Eggs | |
| | | | | Amphibian | Sea urchin |
| Elevated sedimentation coefficient in strand-separating solvents relative to separate single strands. | + (*13*) | + (*14*) | + (*16*) | + (*17*) | + (*18*) |
| Conversion to slower-sedimenting circular DNA by one or more single-strand scissions | + (*24*) | | + (*16*) | + (*17*) | |
| Elevated $pH_m$ (midpoint of $p$H melting curve in alkaline CsCl) relative to $pH_m$ of DNA with single-strand scissions | | | | + (*17*) | |
| Dip in the sedimentation velocity-melting curve | | | | + (*17*) | |
| Elevated $T_m$ (midpoint of melting temperature) relative to $T_m$ of DNA with single-strand scissions. | + (*15*) | | | | |
| Lower capacity to bind the intercalating dye ethidium bromide (at high dye concentrations) than DNA containing single-strand scissions. | + (*15*) | | + (*37*) | | + (*18*) |
| Highly twisted (supercoiled) circular molecules in electron micrographs. Depending on conditions, these circles may also appear loosely coiled or open. | + (*15*) | + (*14*) | + (*16*) | + (*17*) | + (*18*) |

Methylated bases have not yet been reported in mitochondrial DNA, presumably because they usually occur in very small quantities. Most, but not all, DNA's contain methylated bases. Several types of chloroplast DNA contain undetectable quantities of the methylated base 5-methylcytosine, in contrast to the corresponding nuclear DNA's which have this base (*43*). We are now studying DNA methylation in mitochondria and nuclei of L cells, and differences between the two types are indicated. The significance of methylation of DNA is not yet clear. Methylation of DNA in the bacterium *Escherichia coli* has been linked with DNA replication, although methylation is not a prerequisite, and methyl-deficient T2 bacteriophage was found to have normal biological properties (*44*).

## Synthesis, Turnover, and Replication of Mitochondrial DNA

Do mitochondria have the enzymatic equipment to synthesize organelle-specific DNA on templates of mitochondrial DNA? What is the mechanism of replication of mitochondrial DNA and its relation to mitochondrial duplication? Does the nucleus play a role in the control of mitochondrial DNA synthesis and organelle replication?

Some of the answers to these questions are beginning to emerge in part. Earlier autoradiographic studies, showing that the DNA precursor thymidine is incorporated into regions of the cytoplasm corresponding to mitochondria (*5*), have led to biochemical studies of the incorporation of deoxyribonucleosides into mitochondrial DNA in vivo (*2*, *5*, *45–47*) and of the incorporation of deoxyribonucleoside triphosphates into the DNA of isolated and purified mitochondria in vitro (*24*, *48–51*) (see Fig. 5). The latter incorporation is dependent on the presence of magnesium ions and all four deoxyribonucleotides; the activity is generally not affected by added deoxyribonuclease, which does not penetrate into intact mitochondria, or by additional DNA primer, but it is inhibited by acriflavine, actinomycin D, and some other compounds. Mitochondrial DNA polymerase of rat liver has also been obtained in a soluble fraction, and it appears to differ from nuclear DNA polymerase in its requirements for divalent cations and its response to

added native and denatured DNA primers (*49*). That the deoxyribonucleotides are incorporated into the interior of the DNA molecules, is suggested by end-group analyses (*50*) and by the isolation of labeled, covalently closed circular DNA from the assay mixture (Fig. 5). The latter finding not only suggests that a DNA polymerase was active in mitochondria but also that there may be a ligase present which covalently closes polynucleotides, similar to that described in bacteria (*52*). Additional experiments, however, are needed to demonstrate directly the presence of a ligase and to show that the observed incorporation of deoxyribonucleoside triphosphates into covalently closed circular DNA is not due to a DNA repair mechanism functioning in mitochondria as it does in bacteria. The net synthesis of mitochondrial DNA in vitro per hour is of the order of 0.5 to 2 percent of the DNA present in the organelles. It is not known, however, how functionally homogeneous the mitochondrial population is in the test tube. Also, the presence of deoxyribonuclease in mitochondria may affect the integrity of the product.

An important problem in the study of net DNA synthesis of mitochondria in intact cells is the occurrence of turnover of mitochondrial DNA, which reflects the dynamic state or the assembly and degradation of the molecules. In the in vivo studies cited above, the specific activity (incorporated radioactive DNA precursors per microgram of DNA) of mitochondrial DNA is higher than that of nuclear DNA. The ratio of specific activity of mitochondrial DNA to nuclear DNA tends to be higher (up to about 50) in slow-growing than in fast-growing cells. Correspondingly, mitochondrial DNA of slow-growing tissues turns over more rapidly or has a shorter half-life than mitochondrial DNA of fast-growing tissues like young livers or certain hepatomas (*46*). It has therefore been suggested that the first response to an increased rate of cell division is a greater stability rather than a higher rate of synthesis of mitochondrial DNA; as the rate of cell division gets higher, the rate of DNA synthesis also increases (*46*). The difficulty in these experiments is that it is not known whether the number of mitochondria per cell or the DNA per mitochondrion changes, or what percentage of the mitochondrial and cell population contributes to the analyses. It was shown, however, that at least one other mitochondrial component, phospholipid, turns over at the same rate as mitochondrial DNA (*53*).

The time of synthesis of mitochondrial and nuclear DNA relative to the cell division cycle has been studied in several nucleated microbes and in cultured mammalian cells. In all cases studied thus far, mitochondrial DNA synthesis has proceeded independently of nuclear DNA synthesis (*5, 54*). Although the bulk of the evidence is in favor of a periodicity of mitochondrial and nuclear DNA synthesis, a few examples suggest continuous synthesis of mitochondrial DNA. It is quite reasonable to assume that in diverse cell types there is no complete uniformity in the pattern of synthesis of DNA. An example of periodicity of mitochondrial DNA synthesis has been illustrated in electron microscopic autoradiographs of L cells (*5*). Most cells corresponding to the expected number of cells in the S-phase of growth showed radioactivity of the labeled DNA in the nucleus alone, and not in the cytoplasm, whereas a small percentage of cells was labeled in the cytoplasm only, on or near mitochondria. It will be important to find out what factors control the onset and rate of nuclear and mitochondrial DNA synthesis, and what is the degree of autonomy of mitochondrial DNA synthesis and division.

Studies of the replication mechanism of mitochondrial DNA in *Neurospora* have been reported to be consistent with a semiconservative mechanism (*39*). In this mode of replication (which appears to be universal), each strand of the duplex DNA replicates to form a parent-progeny hybrid in the first generation. Shifts in buoyant densities of DNA after transfer of cells from $N^{15}$ to $N^{14}$ medium were followed through several cell generations. The density shifts obtained for mitochondrial DNA, compared to nuclear DNA, did not follow the pattern expected for a semiconservative replication as clearly. More organisms should be studied to obtain a more clear-cut picture. The mechanical aspects of DNA replication in mitochondria (initiation points, active or passive unwinding mechanism, and swivel points) may remain obscure for some time. Even in studies with bacteria, which are more amenable to experimentation than mitochondria are, the number of working models available is roughly proportional to the number of investigators making the studies.

## Abnormal Mitochondrial DNA

Mitochondrial function and morphology directly reflects some of the metabolic demands of the cell. The amount and complexity of the internal membranes or cristae increase when the demand for respiratory activity or energy production is high. Many pathological conditions are similarly reflected in altered mitochondrial structure and function. Mitochondrial structure may range from highly dense and complex patterns of cristae in hypermetabolic conditions to degenerating cristae-poor types of mitochondria in many tumors. Other commonly observed abnormalities are giant mitochondria and intramitochondrial inclusion bodies. Relatively few cases, however, have been examined where the structure of the DNA of mitochondria is affected. Mitochondria of ascites tumor cells and some virus-induced tumors (*26, 27*) have been shown by electron microscopy to contain large, abnormally shaped DNA fibers. In ascites tumor cells mitochondrial DNA was found to encircle globular inclusion bodies of the type found also within clusters of virus-like particles in the cytoplasm (*26*). In cells infected with "lipovirus," spongiform bodies were observed in mitochondria and adjacent cytoplasm (*55*). No direct evidence has been reported as yet that DNA viruses or other viruses can multiply in mitochondria or have part of their life cycle in mitochondria. Mature virus particles have been observed, however, in chloroplasts of leaves infected by tobacco mosaic virus (*56*).

The mitochondrial DNA's of certain cytoplasmic petite mutants of yeast have been reported to be undetectable (*57*), reduced (*58*), or to differ significantly in base composition from mitochondrial DNA of wild-type yeast cells (*59*). The complete or partial loss of genetic information in the cytoplasmically inherited petite mutants is reflected in a lack of respiratory enzymes, cytochromes a, $a_3$, b, and c, and a deficiency in some dehydrogenases. These components are specifically located in the mitochondria of normal cells. Electron microscopy has shown that these mutants contain abnormally structured, membrane-deficient mitochondria (*60*). The structure of the altered DNA and the type of mechanism leading to this irreversible change of the mitochondrial population has not yet been clarified. The petite mutation may arise spon-

taneously or is induced by ultraviolet light or by very low concentrations of acriflavine and ethidium bromide (*61*), which are dyes known to intercalate in double-stranded DNA. In studies of the mechanisms of action of these mutagenic agents, it must be learned why methylated acridines also intercalate with DNA and yet are not mutagenic, why mitochondrial DNA is preferentially affected over nuclear DNA (perhaps a permeability problem), and how very low concentrations of dye are mutagenically effective and lead to gross changes in the DNA base composition of the mitochondrial population. It appears that a change in the DNA structure, possibly caused by defects in the DNA replication mechanism (DNA polymerase?), may occur in relatively few mitochondria of the cell or in various mitochondria to different degrees. The altered surviving organelles, for reasons unknown, would outgrow the normal mitochondria in successive generations. It is also unknown whether the mutagenic action of the intercalating dyes actually involves the induction of errors in mitochondrial DNA or the propagation of errors that may normally occur in a minor proportion of the mitochondrial population but that are normally repressed.

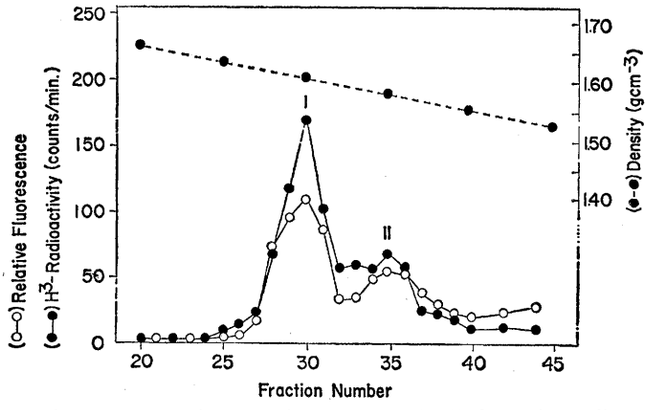


Fig. 5. Covalently closed circular DNA (I) isolated as the reaction product of mitochondrial DNA polymerase activity, after incubation of mitochondria with radioactive DNA precursors [$H^3$-deoxythymidine triphosphate (dTTP)]. The reaction mixture was used according to Wintersberger (*48*). The activity was dependent on the presence of magnesium ions and all four deoxyribonucleotide triphosphates. The reaction rate at 37°C was linear for 15 minutes and then declined. The DNA was isolated from 5 mg of mitochondria after incubation for 10 minutes in the presence of 20 $\mu$c of $H^3$-dTTP per 5 ml (specific activity, 2.8 c/mmole) and centrifuged in a cesium chloride–ethidium bromide gradient (above). The fluorescence of dye-bound DNA and the radioactivity of acid-precipitable DNA were measured. Liver mitochondria from newly hatched chickens were used. The DNA polymerase reaction of L-cell mitochondria also lead to the appearance of label in covalently closed DNA.

## Genetic Function and Coding Capacity of Mitochondrial DNA

One of the most important problems yet to be resolved is the genetic and physiological significance of mitochondrial DNA. To what extent do mitochondria determine their own duplication? Is mitochondrial DNA "nonsense" DNA, or does it code for the production of mitochondria-specific ribosomal and transfer RNA (tRNA) and for the formation of mitochondrial proteins in organelle biogenesis? Does it code for any extramitochondrial proteins? Are all mitochondrial macromolecular components coded for by mitochondrial DNA or are some under the control of nuclear genes? Is the informational content limited to one molecule of DNA? Do additional molecules of DNA in the same organelle duplicate or increase the genetic information?

In studies oriented to test these questions mitochondria have been found to contain most of the components required for the functioning of a specific intramitochondrial genetic apparatus. These components possess distinct characteristics. First, mitochondrial DNA has distinct structural properties and can be synthesized and replicated within the organelle. Mitochondria are also capable of synthesizing RNA (*1–5, 46*). They contain ribosomes and ribosomal RNA's which differ from those of the cytoplasm in size, sedimentation rates, and functional attributes (*62–64*). Mitochondria also contain specific species of tRNA and aminoacyl-tRNA synthetases (*65, 66*) (which catalyze the selective attachment of amino acids to RNA in protein synthesis). Our studies with rat liver mitochondria have shown that these organelles contain many species of tRNA not found in the rest of the cytoplasm and that cytoplasmic synthetases cannot acylate exclusively mitochondrial species of tRNA (*66*). Mitochondrial tRNA was also shown to hybridize with mitochondrial DNA (*67*). Finally, mitochondria incorporate amino acids into protein (*1–5*), in particular into the inner membrane (*68*), and the amino acid incorporation system in contrast to the cytoplasmic system is specifically sensitive to several antibacterial antibiotics (*69*).

The presence of DNA and components of the DNA and protein synthesis apparatus in mitochondria, however, does not in itself prove the genetic function of mitochondrial DNA. The following experimental results, taken together, strongly suggest, however, that this DNA has a genetic role. (i) Mitochondrial DNA in mutants of *Neurospora* was shown to be inherited by the mechanism of maternal inheritance (*39*). (ii) Mitochondrial phenotypes apparently have been transmitted by microinjection of organelles in *Neurospora* (*70*), although the fate of the injected material is unknown. (iii) Certain respiratory mutants of yeast, as discussed earlier, have structurally altered DNA. (iv) Recent evidence suggests the occurrence of recombinational events of mitochondrial DNA in crosses between several strains of cytoplasmic yeast mutants which show resistance to different drugs (*71*). The drug resistance (for example, to erythromycin) of some mutants was shown to be under cytoplasmic genetic control (*72*). (v) Hybridization experiments of nuclear or mitochondrial DNA with mitochondrial or cytoplasmic ribosomal RNA in yeast (*63*) or a particulate RNA in *Tetrahymena* (*64*) suggest common base sequences in mitochondrial DNA with mitochondrial ribosomal RNA. (vi) Hybridization experiments in our laboratory have shown that mitochondria-specific species of aminoacyl-tRNA of rat liver have common base sequences with mitochondrial DNA, in contrast to cytoplasmic tRNA which does not significantly react with mitochondrial DNA (*67*). (vii) Mitochondrial RNA polymerase can be inhibited by actinomycin D and may therefore be DNA-dependent (*24, 25, 46*). (viii) The structural protein isolated from mitochondria of cytoplasmic poky mutants of *Neurospora* contained a slightly different amino acid composition (differences in tryptophan and

cysteine content) from that of the wild-type protein, which suggests that this is a direct consequence of a genetic defect on mitochondrial DNA (*73*).

Although it may be assumed from the above that mitochondrial DNA is genetically active, this small piece of DNA is probably insufficient to account for all macromolecular components of a mitochondrion. A DNA circle of molecular weight 9 to 10 $\times$ $10^6$ (approximately 15,000 base pairs) can code for only about 5000 amino acids or 30 polypeptide chains of molecular weight 20,000 (if we assume that a triplet coding mechanism is universal). The problem must therefore be considered whether individual mitochondria may contain several genetically different DNA molecules or whether nuclear genes code for some mitochondrial structural components. The latter possibility appears most likely. At least one role of the nucleus in the control of mitochondrial biogenesis has been established. It is well known that nuclear genes in yeast control the synthesis of some cytochromes (*3*), and in mammalian cells cytochrome c is at least partially synthesized on cytoplasmic ribosomes and then transferred into mitochondria, although by an as yet unknown mechanism (*74*). The cytoplasmic ribosomal system is under nuclear control and, in contrast to the mitochondrial ribosomal system in the intact cell, it is specifically sensitive to added ribonuclease and to cycloheximide.

The other possibility, that mitochondria contain several genetically different DNA molecules, is less likely. Mitochondrial DNA has been shown to be highly homogeneous in renaturation studies (*16*), although very slight differences among molecules may not be detectable in this manner. The evidence that the number of DNA molecules within a single mitochondrion is highly variable (*15*) suggests that multiple DNA molecules per mitochondrion represent repetition or redundancy of informational content. The occurrence of fusion and fission of mitochondria may be interpreted in favor of repetitive molecules in each mitochondrial nucleoid, although fusion may also imply recombination of genetically different organelles. It is also unlikely (but not yet experimentally tested) that the double-length molecules seen in some mitochondria carry additional significant informational content, because their occurrence in most cell types is variable and rare.

Present evidence strongly suggests that mitochondrial DNA codes for the synthesis of the inner mitochondrial membranes and (at least in yeast) of cytochromes a, $a_3$, b, and $c_1$ (*1, 68*); it has the capacity to code for the formation of mitochondrial ribosomal RNA, multiple species of transfer RNA and possibly some enzymes. It may be calculated that the base sequences which are homologous to the sequences of 20 species of tRNA (molecular weight 25,000) would occupy about 10 percent of the mitochondrial DNA molecule. The corresponding aminoacyl-tRNA synthetases, however, may not fit and may be coded for by the nucleus. Nuclear control probably directs the formation of the outer mitochondrial membrane and many enzymes (possibly aminoacyl-tRNA synthetases, nucleic acid polymerases, methylases, and dehydrogenases).

It will be interesting to compare the informational content of the 5-$\mu$ circles of higher organisms with the linear mitochondrial DNA of yeast, *Tetrahymena, Neurospora*, and plants, as well as with linear chloroplast DNA of plants. These latter types of DNA (except perhaps that of yeast) consist of filaments that are at least twice as long as mitochondrial DNA from higher organisms. It has been suggested before (*3, 5, 6, 8*) that mitochondria and chloroplasts have bacteria-like ancestors in the evolution of the cell. If this were true, the organelle DNA in microbial forms may represent intermediate lengths between prokaryotic DNA and mitochondrial DNA of higher forms. The molecular weights are of the order of $10^9$ for bacterial DNA, 5 $\times$ $10^8$ for mycoplasma, 2 $\times$ $10^7$ to $10^8$ for protozoan and fungal mitochondrial DNA as compared with 1 $\times$ $10^7$ for animal mitochondria. It remains to be seen whether microbial mitochondrial DNA contains more intramolecular redundancy or more genetic information than the 5-$\mu$ circles of higher forms. It is of course unknown whether any redundancy exists also within a 5-$\mu$ molecule or whether the coding capacity of this unit is saturated.

Also it will be interesting to see whether organelles with 5-$\mu$ DNA can be readily integrated into the main (nuclear) genetic apparatus of the cell and whether or how the coding mechanism differs in organisms at higher and lower phylogenetic levels. Indeed it is still conceivable that nuclear DNA contains a blueprint or master copy for the base sequence of a mitochondrion (*1*). Current hybridization tests are not sensitive enough to either prove or exclude this possibility. The extent to which part or all of this hypothetical nuclear blueprint is used may depend on the evolutionary level of the organism, as discussed above, or on the appearance of adverse factors that inhibit the genetic apparatus of the mitochondrial population of the cell. In general, however, the large number of mitochondria in most cells and the probable high degree of redundancy of mitochondrial genetic content confer good chances for survival of the cell.

More refined hybridization, purification, and genetic techniques than now available may soon settle many of these problems.

## Conclusion and Outlook

The structure and physicochemical properties of mitochondrial DNA in a wide range of organisms, and many components and properties of the protein-synthesizing apparatus of mitochondria, have been well analyzed in recent years. It has become highly probable that mitochondrial DNA represents a second genetic system of the cell. It has become clear, however, that mitochondrial DNA does not contain sufficient genetic information to code for all mitochondrial components. The interaction of the nuclear genetic system with that of mitochondria appears to be essential in organelle biosynthesis. It is evident that both lines of research, the identification and properties of mitochondrial components as well as the synthesis, interrelation, and apparent dual control of these components, will require extensive further exploration. The possible role of DNA and RNA methylation, the existence and possible effects of polyamines on nucleic acid metabolism in mitochondria, and specific hormone effects also deserve future attention. The genetic function of mitochondrial DNA will undoubtedly become better understood in eukaryotic microorganisms, where mutants are available, than in cells of vertebrates where mutations may be lethal or are difficult to detect and utilize as a tool.

A potentially rewarding field may prove to be the possible role of the

mitochondrial genetic system in some diseases. Similarities in structure and mechanism of replication of mitochondrial DNA with bacterial and viral systems makes mutability of mitochondrial DNA an important possibility which may lead to pathological changes in the cell. It may become possible to use pharmacological agents that selectively affect mitochondrial functions to influence some pathological conditions. The possible role of mitochondrial functions in normal embryological development and differentiation of cells also deserves extensive investigation.

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