Genetics and Phenogenetics of Mitochondria

Some characteristics of mitochondria are inherited independently of genes on the nuclear chromosomes.

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Mitochondria are membranous, particulate elements of irregular shape, about 3 microns in their longest dimension, found in the cytoplasm of the cells of all eucaryotic organisms. (The eucaryotic organisms have true nuclei; they include all organisms except bacteria, viruses, and blue-green algae.) Mitochondria were recognized about 100 years ago, but relatively little attention was given them by physiologists and biochemists until after World War II, when it first became evident that they were directly involved in aerobic respiration. By the middle of the 1950's it became clear that these organelles are the prime centers of the oxidative pathway which results in the formation of H_2O from O_2 and H and in the associated production of the energy-rich adenosine triphosphate (ATP). They are now generally acknowledged to be the "powerhouses" of eucaryote cells.

Mitochondria have other functions, in addition to their role in aerobic respiration, which are just as pertinent to a thorough understanding of the functioning of the eucaryote cell. Chief among these is the role of assuring genetic continuity of the mitochondria, first postulated as a function over 75 years ago but not demonstrated until recently.

In 1964 Gibor and Granick wrote an article (*1*) which gathered together information from a variety of sources, showing rather convincingly that the plastids and mitochondria of the eucaryotic organisms possess inheritable systems independent of the nuclear system. Since it had been assumed until quite recently that the nucleus was the only organelle in the eucaryote cell possessing genetic material of any consequence, this new emphasis on an old idea stimulated considerable thought and research, leading to some of the matters discussed here.

Although cytoplasmic inheritance had been recognized in plants since at least 1909 (*2*), and a considerable amount of work had been done, particularly in Germany in the period between the two world wars, its significance was underrated by most geneticists, who tended to sweep the matter under the rug. Now, however, it has become abundantly clear that geneticists such as Sonneborn, Michaelis, and Sager, among others (*3*), have been justified in maintaining that other entities in the cell, besides the nucleus, are important in inheritance and in the direction of cell function.

Mitochondria and Cytoplasmic Inheritance

Perhaps the first clear evidence linking mitochondria and the inheritance of certain characteristics has come from the discovery and analysis of the "poky"-type mutants of the fungus *Neurospora crassa* (*4*) and the "petite"-type mutants of the yeast *Saccharomyces cerevisiae* (*5*). It is particularly clear in *Neurospora*, in which the inheritance pattern is most easily studied, that the poky character, which results in a slow growth not affected by supplementation with growth factors, is inherited only through the female (protoperithecial) parent and cannot be transmitted through the male parent. The male parent in *Neurospora* is apparently capable of contributing only a nucleus. This is a happy circumstance which makes *Neurospora* and a number of other fungi like it particularly valuable for the study of cytoplasmic inheritance. In yeast the sexual cycle is initiated by the complete fusion of two haploid cells, which results in a zygote containing a mixture of the cytoplasms of both parents.

Both the poky and the petite mutants have deficiencies in respiratory capacity and definite changes in certain constituents of their mitochondria. Thus, in the poky strain mi-1, cytochromes b and $a + a_3$ are missing, whereas cytochrome c is present in much higher concentration than in the wild-type mitochondria. Additionally, the mitochondrial enzymes cytochrome oxidase and succinic acid oxidase have distinctly lower activities in this strain than in the wild-type *Neurospora* and the content of mitochondrial fatty acids is much higher in the mutant (*6*). Cytochrome and enzyme deficiencies are also found in the mitochondria of petite yeast (*5*), and, in addition, in this strain a drastic reduction in activity of the enzyme L (+) lactate dehydrogenase is accompanied by a rise in D (+) lactate dehydrogenase activity (*7*).

The next step toward understanding the poky mutants came about as a result of an analysis of the "structural protein" of the mitochondria of *Neurospora* (*8*). In these studies the structural proteins of both the wild type and the poky mi-1 mutant were compared with respect to amino acid content and sequence. It is evident that they are different; the structural protein of mi-1 contains a cysteine in place of a tryptophan present in one of the peptides obtained by tryptic digestion of the wild type. A change in structural protein apparently also exists in the petite mutant of yeast (*9*).

The precise status of structural protein is at present not clear, but such protein appears to be a constituent of membranes of all types present in cells, including those in mitochondria. It is not known whether there is a single kind of structural protein or several such proteins. There is some evidence that only one type exists, in the form of a heteromultimer made up of two different polypeptide chains in *Neurospora* (*10*).

A single amino acid substitution is expected from a change in a single codon in a gene coding for a single polypeptide. Hence, one would assume that mitochondria contain a genetic

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apparatus capable of mutating. In mitochondrial mutants of both *Neurospora* and yeast, a change in a specific mitochondrial protein is indicated. The fact that this change is inherited extranuclearly indicates that the mitochondria themselves contain genetic material and perhaps the machinery necessary for protein synthesis. This in fact has been found to be the case. All plant and animal mitochondria so far examined have been found to possess DNA with linear dimensions that average about 5 microns (*11*). In some mitochondria the DNA is organized as circular strands of double helixes. Under the electron microscope these circular "chromosomes" appear very similar to the chromosomes of *Escherichia coli*, and in fact they probably are naked DNA, like bacterial DNA. This means that, in organization, they are unlike the nuclear chromosomes of the eucaryotes, because in these the DNA is ordinarily associated with large amounts of protein.

Furthermore, protein biosynthesis by isolated mitochondria from a number of organisms, including mammals, yeast, and *Neurospora*, has been demonstrated (*12*). The protein biosynthetic machinery of the mitochondria is reasonably independent of, and separate from, the biosynthetic machinery in the extramitochondrial cytoplasm.

In view of these findings it is not surprising that mitochondria can carry genetic alterations which are inherited and expressed independently of the nucleus. Mitochondria are at least quasi-independent entities capable of coding for protein contained in them. We must assume, however, that they are capable of coding through their DNA for only part of their protein, because a DNA strand 5 microns long cannot be expected to code for more than about 50 polypeptide chains of molecular weight 15,000 to 20,000, using a triplet code. In addition, mitochondria are known to contain proteins which are under the control of structural genes in the nucleus. This is discussed more fully below.

Direct support for the conclusion that certain characteristics of the mitochondria are independent of the nucleus has been obtained by transferring washed mitochondria by microinjection from one strain to another (*13*). A mutant strain of *Neurospora*, strain abn-1, which genetic analysis showed to be the result of an inherited change outside the nucleus, was used in these experiments (*14*). When nuclei or DNA fractions from abn-1 are injected into the mycelium of wild-type *Neurospora* by a microinjection technique, no evident change is noted in the wild type. However when mitochondria are isolated from abn-1 and injected into wild-type *Neurospora*, the injected strain takes on the characteristics of abn-1, showing changes in growth rate, morphology, reproductive characteristics, and cytochrome pattern. In addition, mitochondria from the poky strain discussed above have been injected into the wild type, with the result that the wild recipients assume the poky phenotype (*15*).

It is certain that mutations can originate in mitochondria and are transmitted by mitochondria, and also that a mutant mitochondrion, once it has arisen, will express its characteristic and may drive out, or at least dominate, other types of mitochondria—in the above examples, wild-type mitochondria. It is obvious that mitochondria must reproduce or replicate themselves at least partially.

Biogenesis of Mitochondria

The idea that mitochondria replicate themselves quite independently of the nucleus is an old one dating back at least to the 1890's. Only in the last 5 or 6 years, however, has experimental confirmation of this idea been achieved. It was first demonstrated in *Neurospora* that a pulse of radioactive choline fed to a choline-requiring strain in the log phase of growth rapidly took up the choline into the mitochondria (*16*). If the mitochondria are observed after the pulse, when nonradioactive choline is provided, it is found that the subsequently derived mitochondria all have the same amount of radioactivity distributed among them. New mitochondria are derived from old mitochondria, presumably by fission after a period of growth. Actually, what appear to be stages in fission have been observed in electron micrographs of the mycelium of *Neurospora* (*17*) and other organisms. Indeed, an analysis of the mitochondria in *Neurospora* mycelium of different ages indicates that the mitochondria divide synchronously in a given part of the mycelium (*17*).

For some years it had been assumed that mitochondria could arise completely *de novo* from nonmitochondrial material. This assumption was based in part on the observation that in yeast grown in anaerobic culture no mitochondria are evident, but that when there is a shift to aerobic respiration, mitochondria appear. Now, however, it is fairly certain that "promitochondria" persist during the anaerobic phase, presumably in the form of mitochondrial DNA and membrane material which is assembled, along with other elements, in the cell (*18*).

The genesis of mitochondria from previously existing mitochondria is in accord with a great deal of cytological observation both old and recent. For example, the mitochondrial material in the spermatogonial cells of certain scorpions has been shown to be divided equally among the four spermatids (*19*), and on numerous occasions cytologists have observed, by time-lapse photography, what appear to be mitochondria dividing during cell division.

The fact that mitochondria are about the size of bacteria has caused many observers to hypothesize that they were originally bacterial endosymbionts which entered in symbiosis with another type of cell in the remote past and began the eucaryotic line of organisms (*20*). The discovery of DNA in mitochondria having a bacterial type of organization has, of course, strengthened the possibility that mitochondria were once bacterial invaders. If they were, then one should indeed expect them to arise only from preexisting mitochondria.

Notwithstanding the evidence concerning the origin of mitochondria from mitochondria, it is fairly certain that not all the mitochondrial protein is synthesized intramitochondrially by way of the mitochondrial protein-synthesizing system. For one thing, as pointed out above, there would appear to be insufficient DNA in the mitochondrial chromosomes to account for all the different types of proteins in mitochondria.

Origin of Mitochondrial Protein

Evidence from studies on yeast in particular indicate that mitochondrial protein is formed in two ways: (i) by synthesis within the mitochondria, by the protein-synthesizing machinery there present, and (ii) by synthesis outside the mitochondria, presumably in association with the endoplasmic reticulum, or microsomal fraction of the cytoplasm. The discovery that the anti-

biotic chloramphenicol, at low concentrations, inhibits protein synthesis in the mitochondria of yeast and other organisms, but apparently not in the cytoplasm outside the mitochondria, provides a tool for determining by which system a specific protein is made (*21*). The synthesis of cytochromes a and b of yeast is sensitive to chloramphenicol, but the synthesis of cytochrome c is not (*22*). Hence it may be tentatively concluded that cytochromes a and b are made in the mitochondria while cytochrome c is not. Direct evidence that cytochrome c is synthesized in the microsomal fraction of the extramitochondrial cytoplasm, under the direction of the nuclear genes, has been found by a number of workers (*23*) and at this time seems to be quite conclusive. The amino acid sequence of cytochrome c in yeast is demonstrably altered by mutations in the yeast nucleus (*24*).

The two protein-synthesizing systems, cytoplasmic and mitochondrial, seem to be quite different, at least in yeast and *Neurospora*. The mitochondrial ribosomes, for example, are similar to 70*S* bacterial ribosomes rather than to the 80*S* ribosomes found in the cytoplasm of all eucaryotes (*25*). Furthermore, at least some of the transfer RNA's and activating enzymes involved in protein biosynthesis in *Neurospora* are specific for mitochondrial synthesis (*26*). These observations are consistent with the finding that the two systems are sensitive to different antibiotics. However, one must be careful about making firm generalizations about differential antibiotic effects. While chloramphenicol inhibits protein biosynthesis in the mitochondria from rat liver, it does not do so in the mitochondria from rat brain cortex (*27*).

The results obtained during the last few years by a considerable number of independent investigators have consistently supported the view that mitochondrial protein is synthesized by two different biosynthetic systems. The biogenesis of mitochondria involves, first, a synthesis of certain insoluble proteins, such as structural protein, by the system within the mitochondria and the subsequent integration of soluble protein, such as cytochrome c, synthesized in the cytoplasm (*28*). Nothing, however, is known about the mechanism of assembly of components made within and without the mitochondria into a complete mitochondrion.

Interrelationships

Since mitochondrial protein is derived from at least two sources, one of them being protein that is coded for in the nucleus, the nucleus of the cell determines part of the structure and function of the mitochondria. But this influence over the mitochondria extends beyond the mere alteration of mitochondrial protein that is coded for in the nucleus. For example, a nuclear gene mutant of *Neurospora*, strain C115, has no cytochrome a, a small amount of b, and significantly more c than the wild type (*29*). If cytochrome-a synthesis in *Neurospora* is directed by a mitochondrial gene, as appears to be the case in yeast, then it is evident that a nuclear gene can have a quite direct effect on the expression of a mitochondrial gene. In addition, when the nuclear gene *f* is introduced into poky cytoplasm it suppresses the poky phenotype, causing it to grow at approximately the same rate as the wild type even though the defects in the cytochrome system remain unchanged (*30*).

There is considerable indirect evidence that the activity of an enzyme in the mitochondrion (or elsewhere, for that matter) is strongly influenced by the configuration of the site of attachment (which may be another protein or a lipid) in or on a membrane. If binding is not normal, the enzyme may be inactive in vivo even though it may be active in vitro. Two factors must be considered relative to this: the wild-type enzyme's activity may be impaired by a change in configuration of the site to which it attaches, or the enzyme may be altered by a single amino acid substitution that renders it inactive when attached to a wild-type site even though it is sufficiently unchanged to have wild-type activity in vitro.

The malic dehydrogenase mutants of *Neurospora* illustrate this second possibility. Malic dehydrogenase exists in *Neurospora* and other eucaryotes in two forms, mitochondrial and cytoplasmic, which are separable by starch gel electrophoresis (*31*). Mutations in at least two nuclear genes cause alternations in the mitochondrial form of the enzyme (*32*), so it must be assumed that the structural genes reside in the nucleus. When the malate dehydrogenases from the various strains are extracted and their activities are determined, it is found that the specific activities of the enzyme from the mutants is 20 to 90 percent of the wild-type specific activity. Despite this, all of the mutants are essentially complete auxotrophs, acting as if they contained no effective malic dehydrogenase activity. Second, when the mutant dehydrogenases are solubilized they are found to have about the same affinities for malate as the wild-type enzyme, as determined by their Michaelis constants (*33*). In the intact mitochondria or in the presence of wild-type mitochondrial membranes, however, the affinities are drastically altered downward (*34*). It can be assumed, then, that the mutant dehydrogenases are relatively inactive at their site of operation in the mitochondria. When they are extracted and in solution, their activities are artifactitious. It is indeed best to think of all enzymes operating in vitro as artifacts in the sense that their actual activity in vivo can probably be known only through study in situ. This unfortunately introduces the uncertainty principle, making life just as difficult for the biologist as it is for the physicist.

Returning now to the possibility that an enzyme's activity may be changed by an alteration of the site to which it binds, we find several examples which seem to fit this situation. One of the important enzymes involved in oxidative phosphorylation in the mitochondria is adenosine triphosphatase, which catalyzes the terminal step,

$$\mathrm{ADP} + \mathrm{X} - \mathrm{P} \leftrightarrows \mathrm{ATP} + \mathrm{X}$$

(ADP and ATP are, respectively, adenosine di- and triphosphate). In the intact mitochondrion this enzyme is bound to the inner membrane, where it may be seen under the electron microscope and identified as the unit elementary or submitochondrial particles, which appear to be spheres about 90 angstroms in diameter (*35*). Ordinarily, in the intact state, adenosine triphosphatase is stable with respect to cold, and highly sensitive to the antibiotic oligomycin. However, when it is solubilized it becomes cold-labile and insensitive to oligomycin (*36*). Hence, its properties differ radically depending on whether it is or is not membrane-bound.

It has been reported that in both the petite mutant of yeast (*37*) and the poky mutant of *Neurospora* (*38*) the adenosine triphosphatase associated with the mitochondria is cold-labile and insensitive to oligomycin, corresponding to that removed from the mitochondria

and solubilized. However, if the adenosine triphosphatase of the petite mutant of yeast is solubilized and then bound to the mitochondria of the wild type, it becomes cold-stable and strongly inhibited by oligomycin. Thus it is highly probable that this enzyme is not altered in its basic structure by the petite mutation. Its primary structure is probably identical to that of the wild-type enzyme, but in the altered petite mitochondrion its properties are changed, since the "structural protein" of the petite mutant is altered (*9*). The same explanation probably applies to the adenosine triphosphatase of poky *Neurospora*, in which, it is also quite certain, the structural protein is altered.

A related situation is seen in the effects of the poky, as compared to the wild-type, structural protein on the malic dehydrogenases from the various *Neurospora* strains. As noted above, the malic dehydrogenases from the mutant strains demonstrate quite different Michaelis constants (K_m) depending on whether they are free in solution or bound to mitochondria. It is also true that the wild-type enzyme has about the same affinity for malate regardless of whether it is free or bound to wild-type structural protein. However, if it is bound to poky structural protein its affinity for substrate is lowered tremendously (K_m goes from 0.72 to 500) (*34*).

It would seem from these various observations that alteration of the structural protein of poky *Neurospora*, and also probably of petite yeast, causes widespread changes in the activities of the enzymes contained within the mitochondria. Recent evidence also indicates that the effects may be even more extensive than this, because it appears that at least part of the structural protein originally thought to be confined to mitochondria is also found in the other cell membranes—that is, the microsomes—and in a nuclear, as well as a supernatant, fraction obtained after centrifuging nuclei, microsomes, and the mitochondria. A comparison of the wild-type and poky structural protein in these various membranous components indicates that all are similarly affected in poky (*34*). Hence it appears that a mutation in a mitochondrion may cause changes in proteins outside the mitochondria.

Although the mitochondrial and cytoplasmic protein-synthesizing systems seem to be quite different, and independent, as emphasized above, there is a possibility that an exchange of nucleic acids occurs such that some mitochondrial protein is synthesized in the mitochondria by way of an RNA template provided from the nucleus. There is evidence that some of the messenger RNA in the cytoplasm of HeLa tumor cells arises from the DNA of the mitochondria rather than from that of the nucleus (*39*). This may be a condition found only in neoplastic cells, but it does introduce another substantive reason for being open-minded about the relative degree of independence of the mitochondrial and cytoplasmic systems.

Heterogeneity of Mitochondria

Are all the mitochondria in a given cell or a given organism necessarily always the same? The answer would seem to be "probably not," since mitochondria can mutate. One should therefore expect to find a condition of mitochondrial genetic heterogeneity similar to heterokaryosis or nuclear heterogeneity in fungi. Such heterogeneity or mitochondrial polymorphism has been found in several instances. In yeast it has been shown that two kinds of mitochondria may exist in the same cell, those that contain cytochrome c oxidase and those that do not (*40*). Normal rat-liver mitochondria distributed by size following centrifugation in a linear density gradient showed a heterogeneous distribution of several of 12 mitochondrial enzymes assayed (*41*). However, these phenotypic differences do not necessarily represent genotypic differences in mitochondria, especially in the case of rat liver. They may represent differences in stages of development of the mitochondria, or environmental differences in different parts of the same organ. For example, it is known that the mitochondria in the pericentral cells of the liver are considerably smaller than those in the peripheral cells (*42*). And it has been shown that the density and composition of *Neurospora* mitochondria can be varied substantially by changing the levels of certain growth factors required by auxotrophic mutants (*43*).

A significant difference has been found between rat-brain mitochondria and liver mitochondria from the same animal. As mentioned above, the brain mitochondria are not sensitive to chloramphenicol (*27*). This finding is in direct disagreement with the effect of this antibiotic in inhibiting protein synthesis in liver and fungal and plant mitochondria. The fact that acetoxycycloheximide, the inhibitor of cytoplasmic protein synthesis, also inhibits protein synthesis in the rat-brain mitochondria indicates that in these mitochondria the "usual" mitochondrial biosynthetic mechanism has been replaced by the cytoplasmic type (*27*). Here we have an indication of a heterogeneity that is difficult to explain without assuming that in the rat egg there are at least two kinds of mitochondria which become segregated during development. Otherwise we must assume a rather drastic type of transformation of genotype, or assume that in some way the cytoplasmic biosynthetic-type pathway can be introduced into the mitochondria during their development.

Another remarkable variety of mitochondrial heterogeneity has been described, in maize (*44*), which presents aspects that are difficult to explain on the basis of the simplistic assumption that mitochondria are relatively independent functional entities which reproduce themselves by a simple fission. Two inbred strains of maize (*Zea mays*), Oh9 and wf45, have been studied with respect to the density of their mitochondria, as determined by centrifugation in a linear sucrose gradient. It was found that the Oh9 mitochondria sediment as two distinct bands, and that the wf45 mitochondria sediment as a single band corresponding in density to one of the Oh9 bands. Strain Oh9 demonstrates a polymorphism in its mitochondrial population, whereas wf45 does not, as measured by density.

The hybrids between strains Oh9 and wf45 are heterotic, showing more vigorous growth than the parent inbred strains. Linear gradients of the mitochondrial fraction from the hybrid show three distinct bands after centrifugation, two corresponding to those found in the parents, and a third band of intermediate density, not found in either parent. The origin of mitochondria of the third type is not certain, but conceivably they could arise by fusion of the two other types to yield particles of intermediate density. In any event some type of complementation between the parental mitochondrial forms is indicated, because the phosphorous-oxygen ratios

obtained with mitochondrial fractions from the inbred parental lines wf9 and Oh45 were 1.45 and 1.29, respectively, while the ratio for the hybrid was 1.72. This increase in the hybrid in the rate of oxidative phosphorylation correlates with the increase in the rate of oxygen uptake, and in the rate of growth of the hybrid as compared to the parental strains. Indeed it would appear that the vigor manifested by the hybrid is the result of more "vigor" in hybrid mitochondria. These finds are of the utmost importance, because they demonstrate not only polymorphism of mitochondria but the fact that different mitochondria, like different allelic genes, may produce "heterozygous" combinations superior to either parent.

Genetic Implications

The existence of heterogeneity in mitochondria within the same species of eucaryote has wide implications in biology, and causes us to change some of our old dogmas and perhaps replace them with newer ones.

For many years it had been tacitly assumed by most cell biologists and geneticists that mitotic cell divisions ordinarily result in the formation of two identical daughter cells from the mother cell. It should now be evident that this assumption is not valid, if cells carry heterogeneous populations of mitochondria, unless there is some mechanism to insure that each daughter receives one of each type of mitochondrion, just as each receives one of each type of chromosome in ordinary mitotic karyokinesis.

One might expect, then, that so-called monozygotic identical twins will not always be identical with respect to their mitochondrial genetic material. An interesting test of the degree of identity of quadruplet nine-banded armadillos (*Dasypus novemcinctus*) has been made recently, and a wide range of biochemical differences was found within each quadruplet set (*45*). This would be of no great significance were it not for the fact that female armadillos typically give birth to monozygotic quadruplets (*46*). Obviously this is a matter which should be pursued further in the laboratory. It is of significance in connection with differences not only between individuals but within individuals. For if mitochondrial segregation or sorting out can occur in the early stages of development, it may also occur in later stages, with the result that the individual will demonstrate a mosaicism with respect to mitochondria. Sorting out or segregation of plastids is well demonstrated in plants (*47*), and one should expect the same segregation for mitochondria. A mathematical analysis has been given this phenomenon in connection with plastid inheritance (*48*).

Coupled with the possible consequences of the sorting out of a heterogeneous population of mitochondria originally present in an egg cell are the various possibilities relating to the origin of the mitochondria in the fertilized egg. Leaving aside organisms such as *Neurospora* and yeast, which do not possess eggs in the strict sense but possess pronuclei of opposite mating type which fuse, with or without cytoplasmic accompaniment, from both sexes, let us consider primarily the higher multicellular eucaryotes, which do have true gametes.

It has been known for many years that the eggs and sperm in all animals and plants studied invariably contain mitochondria or elements derived from mitochondria (*49*), and that generally the mitochemical elements of the sperm enter the egg. A number of reports in the literature indicate that, in some species of animals, such as the worm *Nereis succinea,* the midpiece of the sperm containing the mitochondrial material does not enter the egg at fertilization (*50*). In addition, it is not certain that, in plants, the mitochondria of sperm or pollen tube enter the egg. Notwithstanding these exceptions or uncertainties, it is known that in many, if not most, animals the father contributes mitochondria to the egg, which thus begins its course of cleavage with mitochondrial contributions from both parents. It follows, then, that, if mitochondria sort out during spermatogenesis, sperm may vary considerably in their mitochondrial content. Hence, even if the unfertilized eggs are uniform with respect to their mitochondrial content, the fertilized eggs might vary considerably in this respect.

Summary

The mitochondria of the eucaryotic organisms contain DNA and a protein-synthesizing system distinct from the protein-synthesizing system of the extramitochondrial cytoplasm. This confers upon them a degree of genetic continuity independent of the nucleus. Through this DNA-RNA system they synthesize certain proteins characteristic of them and perhaps of other membranous structures in the cell. The DNA of the mitochondria may mutate and cause changes in these proteins, which are inherited cytoplasmically. These changes in mitochondrial proteins may have widespread effects in the total functioning of the eucaryote cell, which in essence contains *at least* two genetic systems controlling its functions.

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Insect Olfaction: Deciphering System for Chemical Messages

Receptor cells beneath a porous cuticle are highly sensitive and specific to odor molecules.

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The term stimuli, as applied to living organisms, may be thought of as those influences that originate in the interior or exterior environment which elicit a biological reaction. Chemical stimuli, broadly considered, are those chemical influences to which at least one organism reacts. If we confine our consideration to animals, we find that there are two fundamentally different chemosensory mechanisms.

General chemosensitivity is a slow response which usually extends to the body surface and some inner organs of the body after exposure to a relatively high concentration of harmful chemicals. In most cases, this system is protective and serves to counteract the destructive effects of irritating substances.

Receptor sensitivity is a faster response. In this case the receptor cells or their dendritic endings are excited by a relatively narrow spectrum of adequate compounds to which the cell is specific. As a rule, these cells are moderately, or in some cases extremely, sensitive. Out of the receptor response, a nervous message is formed which travels by way of the afferent nerve fibers to the central nervous system. As in all receptor systems, basically similar receptor systems are found in related groups of organisms; however, some striking similarities have developed in nonrelated species as the result of functional adaptations to the environment. Such a receptor system comprises and limits the chemical world in which an organism lives. These worlds differ for different organisms owing to qualitative and quantitative differences in their chemosensory systems.

Taste and Olfaction

The transfer of chemical information requires a chemical source, a medium of transfer (air or water), and a receptor. We here distinguish olfaction from taste for man and the other vertebrates (*1*). Olfactory stimuli elicit a response in the nasal receptors; taste stimuli elicit response in the taste cells of the mouth cavity and, as in some fish and amphibia, also in the moist skin of the body surface.

The transfer medium of the taste stimulus is always water. The qualitative range of stimuli to which a taste cell responds is narrow, and the number of taste qualities is small. In man we recognize four tastes: sweet, sour, bitter, and salty. In the lower vertebrates, the number of different taste qualities may be greater.

The qualitative range of odor stimuli in most organisms is very great. No satisfactory psychophysical or physiological system of classifying odor qualities has been developed. The classical question here is how the sense of smell differentiates among a very large number of odorants (*2*).

Are there in the chemoreceptor systems of invertebrates modalities analogous to those in the olfactory and taste systems of vertebrates? Yes, at least in insects. Behavior tests associated with the localizations of gross anatomical features have suggested the presence of such capacities. Local electrophysiological recordings from the sense organs of insects revealed that the analogy with the vertebrate system is rather close. Taste stimuli in insects are waterborne compounds of a limited range of qualities, some of which are the same as the taste stimulants found in vertebrates. Olfactory stimuli in insects are either air- or waterborne compounds, often very different from one another chemically. As in the vertebrate, taste and olfactory receptors in the insect are anatomically distinct. Taste organs consist of innervated cuticular bristles, hairs, or pegs with an open tip; olfactory organs are of four types as described below. In order to elicit a reaction, the taste cells of insects require stimulating molecules in much higher concentration than do odor receptors.

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