CYTOPLASMIC GENETICS AND EVOLUTION

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PREFACE

All cells of all living organisms, with the exception of bacteria, blue-green algae, and possibly some anaerobic microorganisms, contain more than one sort of inherited organelle or particle that encodes its own genetic message. Nuclei and mitochondria are common to all animal cells, and in addition vertebrate animal cells regularly contain one or more inherited viruses. Inherited bacteria are common in protozoa, and many insects contain a rich fauna of bacteria, sometimes fungi, and, in at least one case, a virus. Plant cells add the plastids and additional cytoplasmic factors that have not yet been identified other than by their genetic effects (plasmon factors). Each sort of particle contains hereditary nucleic acids that encode at least some of the proteins that make up its structure and through which it carries out a biological function.

The major stress in evolutionary thought to date has been on the interactions between the nuclear genes of each organism and its environment. Although it has been recognized that these other sorts of particles have a genetic role, little effort has gone into trying to summarize what that role has been in the context of the evolutionary process. This book is about organisms as multiple genetic combinations and the effects of their interactions on heredity and evolution. The focus is on the fact that the fitness of the organism to survive here is determined by the interactions among its diverse genetic centers, all of which are acted upon during selection and none of which can respond independently of the others. Thus, if a chromosomal mutant is produced that conditions better disease resistance in a plant, natural selection might ordinarily favor that mutant in the population so that it would become the "wild" type. It must pass a test first, however: The enzyme produced by it must not interact unfavorably with the enzymes encoded by the mitochondrial genes

or the plastid genes, for if it does the mutant plant will not survive. A new chromosomal mutant that might otherwise be favorable to the survival of a mouse will be established in a wild population only if it interacts well with mitochondrial gene products and if it does not activate the leukemia or the mammary tumor viruses that are latent parts of the mouse's normal inheritance.

Conversely, a new mutant form of mitochondrion that can divide more rapidly than its brethren might show up in a plant cell and could become the established sort. If the rapid reproduction occurs at the expense of poor interaction with the chromosomal gene products or plastid gene products, the mutant mitochondrion might well destroy the cell and so never survive in the plant. If it survived, however, its effect might be that the plant would not reproduce and hence the mutant mitochondrion would be lost to the population. The evolution of each organelle, therefore, puts constraints on the evolution of each other organelle even as the changes of the environment affect them all.

In trying to understand how this process works, a series of questions has been posed, most of which are the type that would be asked in trying to understand evolutionary processes in nature. How is this particle inherited? What characteristics are encoded in its genetic machinery? Has natural variation been found in strains of this particle in contrasting animal or plant forms? Can it undergo genetic recombination with other particles of the same sort? What happens when the particle from one animal or plant population is combined with nuclear genes of another population? Can the particle function in isolating mechanisms of the host? Is the particle a symbiont or a parasite? How deeply ingrained in the evolution of its host is the particle? Not all the questions can be answered with respect to all sorts of particles, but an effort has been made to comb the literature and to bring together the facts that bear on the answer.

From this emerges a picture of the evolution of higher organisms at two levels. The survival of the animal or plant is dictated by the interaction between its total genetic content and the environment in which it lives. The survival of each competing inherited subcellular particle depends on its relative fitness within the environment of the cell, where the real winner is the one that is maintained without damaging the host in the process.

Several sorts of genetic particles are involved: the mitochondria, plas-

tids, inherited bacteria and viruses, and the as yet unidentified cytoplasmic factors, chiefly of plant evolution. The evolutionary history and force of each type, insofar as we know it, has been outlined and then an attempt made to deduce how each interacts with the products of nuclear genes.

University Park, Pennsylvania
March, 1975

Paul Grun

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PART I / THE MITOCHONDRIA

CHAPTER 1 / INTRODUCTION TO MITOCHONDRIA

Mitochondria are commonly described as organelles situated in the cytoplasm of the cells, and surrounded by two membranes, an outer covering and an inner membrane system ramified into the cristae as sheets or tubules in their inner matrix. A more realistic view might be that the mitochondrion is an organelle in the cisternae of the endoplasmic reticulum having one membrane, the one usually described as its inner membrane. The outer membrane may actually be part, not of the mitochondrion, but of the layer of endoplasmic reticulum membrane that happens to surround it. The following observations support this view.

1. The chemical composition of the outer membrane resembles that of the endoplasmic reticulum (Parsons, 1966; Schnaitman, 1969), whereas the typical mitochondrial functions, including electron transport and the Krebs citric acid cycle, are those of the inner membrane plus matrix (Ernster and Kuylenstierna, 1970). This was determined by isolating mitochondria and then skinning off the outer membrane so that it could be separated from the inner parts for study of its structure and function.

2. Whereas some of the enzymes of the inner membrane are synthesized within the mitochondrion, those of the outer membrane seem to be synthesized outside the mitochondrion. How does one study this? The antibiotic cycloheximide selectively inhibits the cytoplasmic protein-synthesizing machinery. When intact cells of *Neurospora crassa* were raised in a medium that contained radioactive amino acids, normally both the inner membrane and the outer membrane became radioactive, showing that radioactive proteins were built in or on both sorts of membranes. If cycloheximide was added to the cells along with the radioac-

tive amino acids, however, only the inner membranes became radioactive. Since cycloheximide turned off cytoplasmic protein synthesis, it followed that the proteins of the outer membrane were synthesized in the cytoplasm, whereas at least some of those of the inner membrane, being labeled in spite of the presence of the cycloheximide, were synthesized inside the mitochondrion (Neupert and Ludwig, 1971).

3. In recent electron micrographs of mitochondria of a fungus (*Pythium*) (Fig. 1) and a protozoan (*Tetrahymena*) the outer membrane is visibly continuous with the endoplasmic reticulum (Bracker and Grove, 1971; Franke and Kartenbeck, 1971).

Figure 1
Electron micrograph of a cell of the fungus *Pythium*, illustrating the connection between the outer mitochondrial membrane and the membrane of the endoplasmic reticulum. 40,600 ×. (Reprinted from Bracker and Grove, 1971, with permission of Springer-Verlag.)

If, as suggested by Robertson (1961), the endoplasmic reticulum is produced by invagination of the plasma membrane, any material in the cisternae of the endoplasmic reticulum, that is, on the side of an endoplasmic reticulum membrane away from the cytoplasm, might be thought of as being outside of the cell, in extracellular space that happens to be located closely surrounded by the cytoplasm of the cell. The mitochondrion, separated from the cytoplasm by two membranes, is rather well isolated from some of the intracytoplasmic functions (Schnepf and Brown, 1971). It is clear from the many studies of the functioning of mitochondria in the physiology of the cell, however, that this extracellular area is intimately associated with the functioning of the adjacent cytoplasm proper. An extracellular location for mitochondria fits in well

with the suggestions (Chapter 3) that these organelles originated outside the cell and are now established as permanent guests.

The inner mitochondrial membrane and the matrix which this membrane surrounds are, then, the typical mitochondrion area. Much of its physiology is well known (Lehninger, 1964, 1971; Tapley, Kimberg, and Buchanan, 1967; Wainio, 1970), and an extensive literature documents the facts that the insoluble components of the electron transport chain are built into the inner membrane and that the soluble enzymes of the Krebs citric acid cycle are to be found in the matrix. These data show how the mitochondrion functions in energy utilization by the cell. They have been well reviewed and will not be repeated here.

Normal mitochondria, in addition to their functions in energy storage and release, have their own DNA, visible as fine fibrils 1.5–2.5 nm thick (Fig. 2), and a complete assembly of the components that they need for

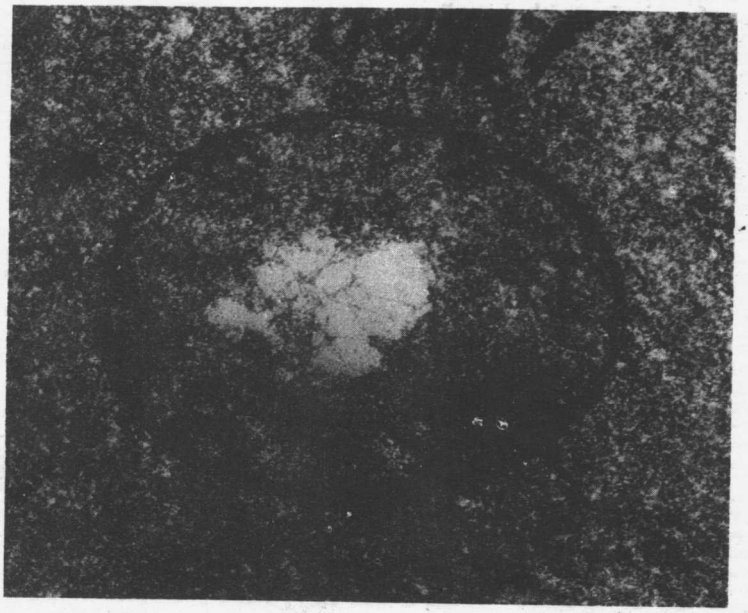

Figure 2
Electron micrograph showing the fine fibrils of DNA in an onion mitochondrion. 75,000 ×. (Reprinted from Nass, Nass, and Afzelius, 1965, with permission of Academic Press.)

their own protein synthesis, division, and maintenance of their genetic system. Ribosomes can be seen inside the matrix of mitochondria in some electron micrographs. They are slightly smaller than those of the cytoplasm and evidently present in larger numbers in mitochondria of embryonic animal tissue than in adults (André and Marinozzi, 1965). To reduce confusion in referring to different sorts of ribosomes, the practice will be followed here of referring to mitochondrial ribosomes as mitoribosomes, to cytoplasmic ribosomes as cytoribosomes (Haldar, Freeman, and Work, 1966), and to chloroplast ribosomes as chlororibosomes. Mitochondria contain their own specialized transfer RNA (mito tRNA), DNA polymerase, RNA polymerase, and probably their own messenger RNA—including, in brief, all the components necessary for mitochondrial protein synthesis. In the discussions of questions, emphasis will be laid on the genetic and evolutionary implications of the functioning of the mitochondrion as a semiautonomous organelle living in and cofunctioning with the cell.

CHAPTER 2 / MITOCHONDRIAL DISTRIBUTIONS DURING GROWTH AND DIFFERENTIATION

HOW MANY MITOCHONDRIA ARE THERE IN A CELL, AND ARE THEY ALL GENETICALLY COMPETENT?

No really definitive counts of the number of mitochondria present in a cell have been made because it is difficult to identify, much less count them in a living cell under the light microscope, where they are streaming about. Counts of stained mitochondria in fixed cells viewed under the light microscope are also difficult because the small specks cannot be definitively identified as mitochondria and because several may appear as one unresolved clump (Avers, 1962). Rough approximations have been suggested based on light and electron microscope views, however, and the figures for higher plant and animal cells have generally ranged from 700–1000 up to an extreme of 200,000 in a large vertebrate egg (Wildman and Cohen, 1955; Avers, 1962; Piko, Tyler, and Vinograd, 1967; Lehninger, 1971).

Single electron micrographs of most eucaryote cells show mitochondria as circles or elongate particles generally a few tenths to half a micrometer (μm) in diameter and 1 or 2 μm long. They have been interpreted as short rods or spheres, though one cannot tell from single electron micrographs whether this view is correct. The three-dimensional structure can be determined by serial sectioning of cells followed by a reconstruction of models from the superimposed pictures, a very laborious procedure in electron microscopy. The few such studies that have been done using *Pityrosporum* yeast (Keddie and Barajas, 1969), *Chlamydomonas* (Arnold *et al.*, 1972), and *Saccharomyces* yeast (Hoffman