INTRODUCTION

In the early years of this century, ideas of partial genetic autonomy for chloroplasts and mitochondria were first postulated by cytologists and geneticists. The initial recognition of cytoplasmic inheritance by Correns and by Baur in 1908 was a description of non-Mendelian inheritance of a factor influencing chloroplast development in leaves. Subsequent work, especially by Renner, Michaelis & Rhodes, established the principles of maternal inheritance of plastid mutations and of plastid autonomy.

Parallel developments in mitochondrial research awaited the discovery of the *poky* mutations in *Neurospora* by Mitchell & Mitchell in 1952 and *petite* mutations in yeast by Ephrussi in 1953. Another decade elapsed before the identification of chloroplast DNA in *Chlamydomonas* by Sager, and the clear demonstration of DNA in mitochondria by Nass and Nass. Since the early 1960s the pace of discovery has quickened; detailed genetic maps and complete sequences have been elucidated, organelle gene products have been identified, mechanisms of protein import and membrane assembly have been probed. A huge area of research has opened up.

The chapters in this book are based on lectures presented as a Seminar at a meeting of the Society for Experimental Biology, held in York during Easter 1987. In this Seminar we restricted ourselves to what is, after all, *the* fundamental problem in the transmission and continuity of organelle-encoded information: the orderly division and segregation of organelles and their genomes during growth and development. A major feature of this Seminar was that it brought together contributions related to animals, plants, yeast and bacteria, and so presented a unique opportunity to look for common themes and illuminating differences between these systems.

Several of the chapters review and reinforce a considerable body of evidence which indicates that chloroplasts and the mitochondria of yeast or plants arise only by the division of pre-existing organelles. However, such evidence is not yet available for the mitochondria of animal cells, and, in view of the existence of complete copies of the mitochondrial genome within the nucleus of human cells (Rosenberger), it would be rash to assume that *de novo* synthesis of mammalian mitochondria never occurs.

What do we know of the physical mechanisms of organelle division? Recently Rhodamine 123 vital staining has become a popular way of tracing mitochondrial movements. The fluorescent images show fast shape changes (< 0.3 s) and often predominantly Brownian movement, but also active locomotion at velocities up to 100 μm/min has been noted. Generation of the necessary forces is mysterious, but attachment to cytoskeletal elements seems a possibility. Similarly, migration and segregation at cell division, or the more precise partitioning required, for instance, during germ tube formation in the dimorphic yeast *Candida albicans*, as studied by Kuroiwa's group, may involve the active participation of contractile elements. Whatley, on the basis of microscopic studies, gives a full description of the visible changes associated with chloroplast division, and provides evidence for the involvement of a contractile system and for control of the polarity of division. Although the nature of the contractile system and the pattern of membrane growth are not yet known, there is the exciting prospect that developments in technology have now reached the point where they should be able to throw light on these important areas.

Information on the dynamics of organelle behaviour is hard-earned. Those working on the division of organelles could be excused for feeling embarrassed by their inability to demonstrate, on demand, an organelle going through the separation step of division. There is no longer any need for such embarrassment, since Leech & Pyke have shown that the separation step is extremely brief for chloroplasts, and so will be observed very rarely even amongst a population of chloroplasts which is actively dividing. Presumably a similar argument could be applied to mitochondria. Even in a system which may appear simple at first sight complexities abound. For instance, the mitochondria of yeast appear in thin sections as discrete, sausage-shaped organelles, many to a cell. This textbook concept was unquestioned until 1973, when Hoffman & Avers showed, in extensive sequences of serial sections, that baker's yeast sometimes has a single, highly branched, giant organelle. Similar three-dimensional reconstructions by Stevens confirmed this complex morphology, as did high voltage electron microscopy of yeasts. Similar findings were reported for other organisms such as Trypanosomes. Cyclic changes between sausages and large networks were shown in *Euglena* and *Chlamydomonas*. These dynamic complexities necessitated reaffirmation that some cell types do in fact contain large numbers of small mitochondria, e.g. the alga *Gonyostomum*, the ciliate *Tetrahymena*, and mammalian liver.

The regulation of chloroplast division has been the subject of intensive study. There is much evidence (Leech & Pyke) that chloroplast growth and division is regulated in such a way that cells maintain a fixed (presumably optimum) 'cover' of chloroplasts in relation to the cell size. This would result in a consistent ability to trap incident radiation, and would be expected to have been favoured by evolution. However, it is not known how this regulation occurs. The mechanisms that regulate

mitochondrial numbers per cell can be probed by the use of drugs that disturb the control loops operating between nuclear and mitochondrial genomes, e.g. chloramphenicol treatment gives *Tetrahymena* with a greatly increased population of tiny mitochondria. Although such results do not allow us to deduce the precise mechanism for regulation of mitochondrial division, they demonstrate that this experimental system has the potential to allow us to identify key steps in the regulatory pathway.

For those who work on organelles there is a reminder of their probable ancestry in the chapter by Donachie, in which he describes the division of bacterial cells. Genes have been identified responsible for almost every aspect of prokaryotic division, and it seems likely that many such genes are also involved in the division of organelles. A major aim of research into organelle division and segregation must be to characterise these genes in as much detail as has been achieved for bacteria.

A recurrent theme of the chapters in this book is the importance of the nuclear genome in the division of organelles and the replication of their DNA. Butterfass shows a strong correlation between nuclear DNA content and the number of chloroplasts per guard cell, and suggests this could be a reflection of the mechanism of regulation of proplastid division (though presumably not of chloroplast division). There is much evidence for the idea that replication of organelle DNA depends entirely on the products of nuclear genes, as in the cases of Albostrians barley (Possingham, Hashimoto & Oross) or petite mutants of yeast (Gingold), which replicate their chloroplast or mitochondrial DNA in the absence of protein synthesis within the organelles. Tilney–Bassett has used genetic analysis to demonstrate a nuclear gene (P_r) which controls plastid inheritance in *Pelargonium*, and whose effects could be interpreted in terms of a nuclear coded plastid DNA polymerase. However, as Bryant makes clear, the characteristic feature of organelle DNA polymerases is how little we know about them.

In the search for other components involved in the replication of organelle DNA, possible candidates include a topoisomerase (tentatively identified by Mills & Baumgartner), and one or more proteins responsible for the attachment of organelle DNA to membranes (Rose). We can expect to see this list grow rapidly over the next few years.

Although research into organelle division and segregation has produced large amounts of information about morphological aspects of these processes, particularly in relation to chloroplasts, it will be clear to the reader that there are huge gaps in our understanding at the molecular level. It is hoped that this volume will encourage even more researchers to attempt to work out exactly how cells manage the remarkable feat of ensuring the maintenance of their organelles throughout growth and cellular division.

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