MOLECULAR CYTOLOGY

Volume 2
Cell Interactions

JEAN BRACHET

MOLECULAR CYTOLOGY

Volume 2
Cell Interactions

JEAN BRACHET
Laboratoire de Cytologie et Embryologie Moléculaires
Département de Biologie Moléculaire
Université Libre de Bruxelles
Brussels, Belgium

1985

ACADEMIC PRESS, INC.
Harcourt Brace Jovanovich, Publishers
Orlando San Diego New York Austin
London Montreal Sydney Tokyo Toronto

ACADEMIC PRESS, INC.
Orlando, Florida 32887

United Kingdom Edition published by
ACADEMIC PRESS INC. (LONDON) LTD.
24–28 Oval Road, London NW1 7DX

Library of Congress Cataloging in Publication Data

Brachet, J. (Jean), Date
Molecular cytology.

Includes bibliographical references and indexes.
1. Cytology. 2. Molecular biology. I. Title.
QH581.2.B73 1985 574.87 84-24418
ISBN 0–12–123371–5 (v. 2. : alk. paper)
ISBN 0–12–123373–1 (v. 2. : paperback)

PRINTED IN THE UNITED STATES OF AMERICA

85 86 87 88 9 8 7 6 5 4 3 2 1

PREFACE

When I was asked to write a second edition of "Biochemical Cytology" (published by Academic Press in 1957), I wondered where my own copy of this old book might be. When it became useless for teaching purposes, it had to leave the desk for some distant bookshelf. I finally found it and put it back on the desk to be subjected to autopsy. My verdict was that only two things retained some value: the Preface, because the author's general philosophy has remained almost unchanged, and the general backbone (the book's skeleton), because cells have remained cells. However, the picture of the cells has become increasingly complex; we know much more about them and understand them better than we did 25 years ago.

Today "Biochemical Cytology" is of little more than historical interest; our ignorance of major facts a quarter of a century ago makes the reading of this old book almost painful. Reading it now does illustrate fashions in science: many facts and hypotheses which were hotly disputed in "Biochemical Cytology" do not arouse the slightest interest today. Because cell biology has undergone a complete revolution, thanks to the explosive progress of knowledge in the field of molecular biology, the book evolved into an entirely new one—hence the title change to "Molecular Cytology." Nonetheless, the general philosophies of both the author and the book have remained unchanged; the Preface of "Biochemical Cytology" remains valid for "Molecular Cytology."

We have attempted in this book to present an integrated version of what is currently known about the morphology and the biochemistry of the cell. There are many excellent cytology and biochemistry textbooks. What remains to be done is the difficult and important task of linking these two sciences more closely together, now that they have so much in common. This is what we tried to do, with the hope that the book will prove useful to advanced students and research workers.

It has been assumed that the reader already knows the fundamentals of descriptive cytology, biochemistry, embryology, genetics, and molecular biology. Our goal will have been reached if the reader enjoys the attempt we have made to show that structure and metabolism are so closely linked together that they cannot be separated.

Special emphasis has been laid on the problems which are most familiar to the author. This will perhaps excuse the apparent imbalance of the book. If too much is said on embryos and too little on cancer cells, it is because the author has spent much of his life working with embryos and has so far not touched a cancer cell.

Emphasis has been given to the more dynamic aspects of cytology, not to detailed description. More is said about nucleocytoplasmic interactions in unicellular organisms and eggs than about the pure description of cytoplasmic and nuclear constituents.

Hypotheses and personal opinions have not been forgotten, for hypotheses, provided that they can be tested experimentally, may become more important than dry facts. Ideas are as vital for scientists as engines for cars or airplanes. Nowadays, some scientists forget that thinking may sometimes be more useful than performing an experiment.

The book has been written directly in English, and the author may not have expressed the ideas and facts as precisely as he would have wished. But what has been lost in subtlety has perhaps been gained in directness and clarity.

The need for a book dealing with biochemical or molecular cytology is obviously much less acute today than it was 25 years ago. At that time "Biochemical Cytology" was not accepted easily by either biochemists or cytologists. I remember vividly a very distinguished professor of anatomy and histology accusing me publicly of having produced a dreadful bastard; my answer was that hybridization can lead to improvement of crops. Today the battle is over, and there are several excellent books dealing with the molecular aspects of cell biology. Some of them, for instance those of Dyson (1978),* DeRobertis and DeRobertis (1981),† and Alberts *et al.* (1983),‡ are textbooks for students; these texts are remarkably complete, clearly written, and illustrated. I used them frequently for the preparation of these volumes, since a scientist remains a student all his life.

This book, like "Biochemical Cytology," is intended for advanced students and for research workers, but it is not an encyclopedia: to aim at completeness is an impossible task in view of the tremendous growth of the scientific literature during the past years. Already the monumental six-volume treatise "The Cell," which was edited 20 years ago by the late Alfred Mirsky and myself, is obsolete and incomplete. The voluminous literature and the specialization of prospective authors have so far precluded a second edition. A second edition would be a giant treatise and, thanks to computer-assisted literature searches, the reader might be crushed under the weight of documentation. If we believe the Preface of Anatole France's "L'Ile des Pingouins" ("Penguin Island"), such a situation has arisen: The author, who is writing a book on the history of the penguins, seeks information from the greatest art critic in the world. His office is filled with files from top to bottom. Finally, A. France, after climbing on the top of a scale, finds the file dealing with penguin art and lets it drop. All the files escape from their

*Dyson, R. D. (1978). "Cell Biology: A Molecular Approach." Allyn & Bacon, Newton, Massachusetts.

†DeRobertis, E., and DeRobertis, E. M., Jr. (1981). "Essentials of Cell and Molecular Biology." Holt, New York.

‡Alberts, B., *et al.* (1983). "Molecular Biology of the Cell." Garland, New York.

boxes, and the critic dies under their weight. His last words are "Que d'Art!" Since many more papers have been published on cell biology than on art in Penguin Island, the utilization of a complete documentation system would have been exceedingly dangerous for the author and boring for the readers.

No computer has been used for the preparation of the present book except an old rusty one, my brain, where lipofuscins and melanins should be expected to accumulate. My major task has been selection, a process which always brings out criticism because it is arbitrary. Most of the selected references are those of recent papers (where the previous literature is summarized) and of review articles published in easily available journals. Despite their number and quality, papers published in specialized symposia have been seldom quoted.

An author should take responsibility for the choice of the topics, the way he deals with them, and the correctness of the references. He should, as much as possible, avoid factual errors. However, the great English biochemist Frederick Gowland Hopkins told me, many years ago, that all textbooks, including his own, are full of errors. Reading through "Biochemical Cytology," which was once praised as "remarkably free of factual errors," I was ashamed to find the book full of errors. They are in general due to the progress of science, which will always move ahead. Errors will accumulate in the present book and, in agreement with Orgel's "error catastrophe theory" of aging (see Chapter 3, Volume 2), they will ultimately lead to its death. An author is also expected to avoid repetition. But there is repetition (perhaps too frequently) in this book, because I believe that important facts should be told more than once and presented again, in a slightly different way, when they are examined from another angle—at least this is a lesson I learned after more than 40 years of teaching and scientific direction of a laboratory.

In short, the aim of the present book is to present a critical and synthetic view, not an encyclopedic description, of living dynamic cells to young scientists. Whether or not this aim has been reached will be decided by the readers themselves.

It is a pleasure and a duty for me to thank all those who kindly agreed to read the manuscript, in particular Professor Werner W. Franke (Heidelberg), who carefully revised the first three chapters. Thanks go also to my colleagues from the University of Brussels, Professors A. Burny, H. Chantrenne, A. Ficq, and P. Van Gansen, who corrected many errors that had escaped my notice. The heavy burden of selecting and preparing the illustrations went to Professors P. Van Gansen and H. Alexandre and Mr. D. Franckx and that of checking the correctness and completeness of the references to Mrs. A. Pays. Last, but not least, my warmest thanks are due to Mrs. J. Baltus, who had the long and unpleasant job of typing the manuscript, and to the publishers for encouragement and patience.

JEAN BRACHET

CONTENTS

CHAPTER 1

NUCLEOCYTOPLASMIC INTERACTIONS IN SOMATIC CELLS AND UNICELLULAR ORGANISMS: GENE TRANSFER IN SOMATIC CELLS

I. GENERAL BACKGROUND

Many of the results summarized in Chapters 3, 4, and 5 of Volume 1 were obtained from fractionated homogenates that are particularly suitable for biochemical studies. However, these studies must be controlled in order to reduce the introduction of artifacts, such as loss or adsorption of enzymes during isolation and fractionation. Careful EM observations are needed to prove that the isolated fractions are identical to the preexisting intracellular organelles: rupture of nuclei or mitochondria, aggregation of cytoplasmic particles, or degradation of macromolecules by hydrolytic enzymes during differential centrifugation of homogenates are dangerous pitfalls. Very careful work is therefore needed to be certain that the factions obtained in this way are cytologically homogeneous.

The limitations of these methods become more apparent when, as in this chapter, interest shifts from mere description of the chemical composition of the various cell organelles to a more dynamic approach: what is the nature of the interactions that occur between the various cell constituents, and between the nucleus and the cytoplasmic organelles, in particular? It is reasonable to combine the various fractions obtained by differential centrifugation of homogenates. For example, Vishniac and Ochoa (1952) studied the biochemical events that occur when chloroplasts are mixed with mitochondria isolated from animal tissues. In an effort to understand the biochemical role of the cell nucleus, Potter *et al.* (1951) and Johnson and Ackermann (1953) have studied the effects of adding nuclei on oxidative phosphorylations in mitochondria. However, such experiments have little value when the nature of the interactions between nuclei and mitochondria in the intact living cell becomes our objective. There is a very serious reason for doubting, in the case of nuclei, at least, the value of experiments performed on mixed fractions recovered by differential centrifugation of homogenates. If we consider as a test of survival for isolated nuclei the capacity of dividing when they are reintroduced into adequate cytoplasm, there is no doubt that, as a rule, they will be quickly inactivated by contact with the outside medium. The experiments of Comandon and de Fonbrune (1939) on the transplantation of a nucleus into an anucleate ameba fragment showed that brief

contact of the nucleus with the outside medium resulted in the "death" and elimination of the transplanted nucleus. The same is true of the embryonic nuclei transplanted by Briggs and King (1953) into anucleate, unfertilized frog eggs (see Chapter 2). However, progress has been made in this field, as in so many others. A method that allows the isolation of "living" somatic nuclei has been devised by Gurdon (1976). If nuclei, such as those described above, are injected into *Xenopus* oocytes or eggs, they can, as we shall see in Chapter 2, either synthesize various RNA species or replicate their DNA.

In order to better understand the biochemical role of the cell nucleus in the living cell, experimental approaches other than homogenization and centrifugation are needed. One approach is found in molecular genetics, but this is such a huge field that an entire book would be needed to provide all of the details. It must suffice here to discuss briefly the exciting gene transfer experiments that are now being done in several laboratories. This chapter will discuss only the interactions that take place between the cytoplasm and the nucleus as a whole, and not the expression of a specific gene. This question will be reviewed when we discuss cell differentiation in Chapter 3.

Two main approaches have been used to study nucleocytoplasmic interactions in somatic cells and unicellular organisms. One is to work on intact cells and to use autoradiography and other cytochemical techniques as the major tools; the other is to compare the biological and biochemical properties of nucleate and anucleate halves obtained by merotomy of cells, protozoa, or eggs.

The classic experiments of merotomy were done more than 85 years ago by Verworn (1892), Balbiani (1888), Klebs (1889), Townsend (1897), and many others on eggs, protozoa, and animal and vegetal cells. They led to an important conclusion, which was emphasized by Mazia (1952) in a review article: "there is not a single case where an activity has not continued in an enucleated cell." However, the life span of an anucleate cytoplasm varies considerably from cell to cell. Anucleate fragments from mammalian cells (cytoplasts) seldom survive for more than a couple of days; on the other hand, anucleate fragments of the giant unicellular green alga *Acetabularia* can survive for as long as 3 months. An anucleate half of an ameba remains alive for about 10 days. In all cases, the life span of anucleate cytoplasm is somewhat shorter than that of its nucleate counterpart, but the difference between the two is never striking, provided they are maintained under the same conditions.

The fact that anucleate fragments of protozoa and eggs survive and retain their biological activities (even ciliary or ameboid motility) for a certain length of time came as a complete surprise to the author when he attended, more than half a century ago, the first lecture of a cytology course given by Pol Gérard. Naively, the author believed that removing the nucleus would be the same as cutting off the head of a man; this led him to study nucleocytoplasmic interactions in

amphibian and sea urchin eggs, as well as in unicellular organisms such as *Amoeba proteus* or *Acetabularia mediterranea*. Similar work currently being done in many laboratories has provided quite satisfactory answers to many of the questions raised by the survival of the anucleate cytoplasm. This work has also put to rest many of the ideas that had been proposed to explain the inferiority of anucleate cytoplasm to nucleate fragments of cells. For instance, Loeb (1899) had proposed that anucleate cytoplasm dies because the cell nucleus is the main center of energy production. This idea was based on inadequate cytochemical evidence about the intracellular localization of respiratory enzymes, and was disproved by work done on the respiration of nucleate and anucleate fragments of eggs and unicellular organisms (and by the already described experiments on the distribution of the respiratory enzymes in centrifuged homogenates). While the question of whether isolated nuclei are capable of restricted energy production remains debatable, there is no doubt that energy production by the nucleus—if it takes place at all—is negligible in comparison to that which occurs in the cytoplasm.

Based on the facts known in 1925, E. B. Wilson concluded that "the nucleus might be a storehouse of enzymes, or of substances that activate the cytoplasmic enzymes, and that these substances may be concerned with synthesis as well as destructive processes." In other words, the nucleus would be a site for enzyme and coenzyme synthesis or accumulation. Stated in such a general way, this idea is no longer tenable although we may still consider the nucleus a storehouse of some important enzymes synthesized in the cytoplasm. This is the case for the polymerases involved in both DNA and RNA synthesis, and probably for the enzymes that control the metabolism of the dinucleotide coenzyme (NAD), the synthesis of which takes place mainly in the nuclei of both liver cells (Hogeboom and Schneider, 1952) and starfish oocytes (Baltus, 1954). In addition, synthesis of poly(ADP) ribose at the expense of NAD and poly(ADP) ribosylation of chromosomal proteins are also nuclear functions. Thus, E. B. Wilson's suggestion remains valid for enzymes involved in dinucleotide and polynucleotide synthesis but can no longer be accepted for the hydrolytic enzymes that are accumulated in the lysosomes. The same criticism holds for Caspersson's (1950) theory, which proposed that the cell nucleus is the main center of protein synthesis. We know (see Chapters 3 and 4, Volume 1) that most, if not all, of the proteins present in the nucleus are synthesized in the cytoplasm; some of them migrate into the nucleus and eventually accumulate there. The question remains as to whether isolated nuclei are capable of *in vitro* protein synthesis. Nevertheless, it is clear that neither energy production nor protein synthesis can be the major biochemical activity of the nucleus. This negative conclusion was reinforced by the biochemical analysis of merotomy experiments; they demonstrated that the nucleus is specialized in nucleic acid synthesis, as we shall now see.

II. WORK ON INTACT CELLS

Autoradiography is a very valuable method for the study of macromolecule synthesis and has the great advantage that it can be used with intact cells. The principle and the methodology are simple (Ficq, 1959): radioactive precursors, usually tritium labeled, are added to living cells, and their incorporation into macromolecules is observed photographically. The classic precursors are [^{3}H]leucine for the study of protein synthesis, [^{3}H]uridine for RNA synthesis, and [^{3}H]thymidine for DNA synthesis. The technique can be used at the EM level in order to visualize, with excellent definition, the localization of newly synthesized proteins or nucleic acids. Autoradiography also has the unique advantage of showing which cells in a heterogeneous cell population are engaged in macromolecule synthesis. However, autoradiography also has its limitations. It does not allow easy measurement of the uptake of soluble precursors. Thus, if as a result of enucleation the uptake of the precursor is increased or decreased, erroneous conclusions might be drawn. In order to obtain quantitative data, therefore, information about the size of the precursor pool and the chemical composition of the newly synthesized macromolecules should be obtained by other methods.

In the early experiments described in "Biochemical Cytology" (Brachet, 1957), treatment of the cells with the appropriate precursors was a lengthy process, and no adequate inhibitors of DNA, RNA, and protein synthesis were available. Our conclusion at that time, therefore, was that "very active RNA metabolism occurred in the nucleus and that protein metabolism, on the other hand, was not necessarily more active in the nucleus than in the cytoplasm." This conclusion was substantially correct, although it was based on weak and sometimes contradictory evidence. The situation became much clearer when many kinds of cells were submitted to short pulses (1 hr or less) with the tritiated precursors. It was found that thymidine and uridine were quickly incorporated into the nuclei, while labeling of proteins began in the cytoplasm. If the pulse was followed by a chase (usually a treatment with the nonradioactive precursor), kinetic analysis showed that thymidine incorporation into chromatin remained unchanged and that it was limited to nuclear DNA. There was no increase in radioactivity unless DNA replication occurred during a long chase. Labeled nuclear RNA, however, moved quickly from the nucleus into the cytoplasm. Conversely, proteins that had been synthesized on cytoplasmic polyribosomes moved into the nuclei. Thus, as a rule, RNAs flowed from the nucleus to the cytoplasm, while proteins moved in the opposite direction. Autoradiography, besides leading to this important conclusion, has largely contributed to the demonstration of a number of facts that have already been discussed in this book, for example, the existence of an S phase during the cell cycle of DNA semiconser-

vative replication in eukaryotes and of sister chromatid exchanges. The great lability of the nuclear RNAs compared to the long half-life of the cytoplasmic ribosomal RNAs and the stability of the nuclear DNA molecules were discovered utilizing a combined approach of autoradiography and biochemical methods. The use of inhibitors of macromolecule synthesis (HU, aphidicolin, actinomycin D, α-amanitin, cordycepin, puromycine, cycloheximide, etc.) combined with autoradiography also played an important role in increasing our understanding of nucleic acids and protein synthesis at the cellular level. Although all of this work is now mainly of historical interest, it is important to recall here that progress in cell biology still requires a combination of biochemical and cytochemical data. Safe conclusions can never be drawn unless the results are obtained with diverse techniques, such as differential centrifugation of homogenates and autoradiography.

Another cytochemical approach that is now of mainly historical interest but that, like autoradiography, has retained its usefulness is the binding to chromatin, in intact cells, of substances that bind selectively to DNA. For example, the fluorescent dye acridine orange (Ringertz and Bolund, 1969; Ringertz *et al.*, 1971) or tritiated actinomycin D (Brachet and Hulin, 1970) have been used successfully for the study of genetic activity in intact cells. Acridine orange gives a bright green fluorescence with DNA and a red fluorescence with RNA. The work of Ringertz and Bolund (1969) has shown that there is a close correlation between the amount of dye bound by chromatin and its genetic activity. The same is true for the binding of [^{3}H]-labeled actinomycin D, which binds preferentially to guanylic acid residues of DNA and can be detected by autoradiography. If chromatin is in a repressed, inactive state, its DNA is covered by chromosomal proteins and there will be little or no binding of acridine orange or actinomycin D. If lymphocytes are activated by treatment with lectins (which leads to increased RNA synthesis before the induction of DNA replication), binding of both actinomycin D and acridine orange markedly increases (Ringertz and Bolund, 1969). On the other hand, there is a decrease in [^{3}H]-labeled actinomycin D binding when embryonic cells differentiate and become specialized in the synthesis of a major protein such as hemoglobin (Brachet and Hulin, 1970). Actinomycin D binding can even be studied at the ultrastructural level (Steinert and Van Gansen, 1971).

Although the techniques discussed in this section may seem obsolete to many readers—particularly since they obviously cannot compete with more specific methods, such as *in situ* hybridization or immunocytochemistry—they deserve mention for two reasons: (1) they played an important role in increasing our understanding of nucleocytoplasmic interactions and (2) they remain a useful first approach (like Feulgen or Unna staining) in the study of nucleic acid distribution and metabolism in intact cells.

III. ENUCLEATION EXPERIMENTS

In the following section, we shall deal with somatic cells in culture (where enucleation can be obtained by treatment with cytochalasin B), with reticulocytes (where loss of the nucleus is a natural process), and with merotomy experiments on protozoa and the giant unicellular alga *Acetabularia*. Merotomy experiments on sea urchin and amphibian eggs will be discussed in the next chapter.

A. Karyoplasts and Cytoplasts

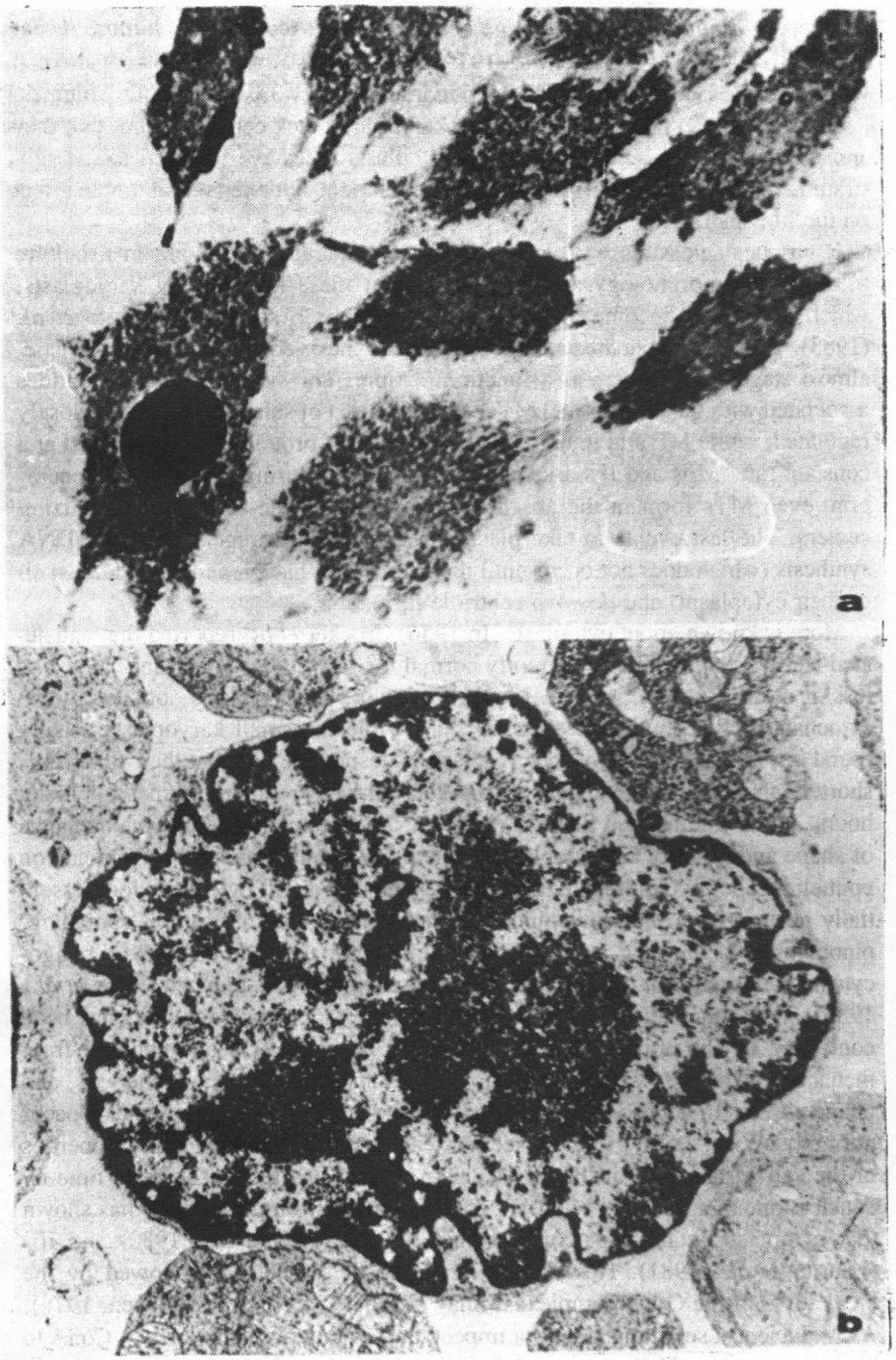
As mentioned in the preceding volume, disruption of the microfilament (MF) cytoskeleton by treatment with cytochalasin B induces, in many cells, the extrusion of the nucleus together with a small amount of cytoplasm (Fig. 1). Low-speed centrifugation helps separate the cytochalasin B–treated cells into small karyoplasts and large cytoplasts, which represent 80–90% of the initial cell volume (Ladda and Estensen, 1970); Poste and Reeve, 1971). Karyoplasts and cytoplasts can be isolated, on a large scale, by taking advantage of their difference in size and density (centrifugation methods) or of the fact that cytoplasts adhere to the substratum better than karyoplasts (Follett, 1974; Wigler and Weinstein, 1974; 1975). When cells undergoing mitosis are treated with cytochalasin B, so-called mitoplasts which are cytoplasmic fragments of dividing cells (Sunkara *et al.*, 1977), can be obtained. Albrecht-Buehler (1980) has described a method, involving cytochalasin B treatment and pipetting, for the preparation of microplasts that represent only 2% of the initial volume of a fibroblast.

Anucleate cytoplasts can be fused to karyoplasts or whole cells of the same or a different species by treatment with uv-inactivated Sendai virus or with polyethylene glycol. Such "reconstituted" cells and the *cybrids* obtained by fusion of a cell with the cytoplast of a different species can survive and divide (Ladda and Estensen, 1970; Veomett *et al.*, 1974; Ege *et al.*, 1974a,b). It has even been possible to fuse cytoplasts from different species, called "heteroplasmons" by Wright and Hayflick (1975). We shall leave cell hybrids and cybrids for a later section of this chapter (Section IV) and limit ourselves here to a comparison between karyoplasts and cytoplasts.

Karyoplasts are formed by the cell nucleus surrounded by a thin, ribosome-containing cytoplasmic layer (Wise and Prescott, 1973). According to Shay *et al.* (1974), they lack centrioles and microtubules (MTs). Karyoplasts remain spherical and die after 72 hr. In the case of myoblasts, karyoplasts do not divide or

---

Fig. 1. Cytoplasts and karyoplasts. (a) Several enucleated Chinese hamster cells (cytoplasts) and a single nucleated cell. (b) An electron micrograph of an L-cell karyoplast surrounded by cytoplasmic fragments. [(a) Prescott and Kirkpatrick, 1978; (b) Lucas (1977).]


a
b

regenerate, dying within 36 hr (Ege *et al.*, 1974a). According to a more recent quantitative study by Zorn *et al.* (1979), karyoplasts represent 10% of the cell volume, possessing 11% of the mitochondrial and only 3% of the Golgi volumes. Zorn *et al.* (1979) have confirmed that karyoplasts lack centrioles, but that they appear, presumably *de novo*, in the karyoplasts that have attached to the substratum. This occurs in only 10% of the population, but exerts a favorable effect on the life span.

It has been possible to select karyoplasts capable of growing in a culture medium; the morphology and biochemistry of these regenerating karyoplasts, which ultimately become complete cells, have been studied by White *et al.* (1983). They found that the pattern of protein synthesis changes in a characteristic, almost stage-specific way as a function of time. The synthesis of polypeptides associated with the nucleus and MTs, as well as that of soluble proteins, is closely regulated, while MF and intermediate filament (IF) proteins are synthesized at a constant rate. MFs and IFs assemble spontaneously during karyoplast regeneration; even MTs form in the absence of visible centriole-associated organizing centers. The last events to take place during karyoplast regeneration are DNA synthesis (which does not occur until the karyoplasts have regenerated almost all of their cytoplasm) and *de novo* centriole formation.

More is known about cytoplasts. In 1956, Crocker *et al.* reported that enucleated HeLa cells remained apparently normal for 40 hr, after which shrinkage and loss of motility were observed. More recent work on cytoplasts obtained by the cytochalasin B technique has shown that, while the small karyoplasts usually round up, the locomotion of the cytoplasts—despite the fact that their survival is shorter (about 48 hr) than that of the karyoplasts—remains normal for many hours. These findings led Shay *et al.* (1974, 1975) to conclude that maintenance of shape and motility are cytoplasmic functions. Similar findings were made on epithelial cells by Goldman *et al.* (1973), who noted that cytoplasts look essentially normal from the viewpoints of attachment to the substratum, spreading, pinocytosis, locomotion, and contact inhibition. In anucleate leukocytes phagocytosis is normal, but digestion is slow and chemotaxis is reduced (Roos *et al.*, 1983). That enucleation has no rapid effect on cytoplasmic activities has been confirmed by the observations of Keller and Bessis (1975) on anucleate fragments of leukocytes, which are capable of normal locomotion, chemotaxis, and phagocytosis. In addition, binding and endocytosis of ConA in cytoplasts occur normally (Wise, 1974, 1976). It seems clear, therefore, that the main properties of the cell surface and of the cytoskeleton remain unaffected for some time by enucleation. In agreement with this conclusion, electron microscopy has shown that cytoplasts retain apparently normal MTs (Brown *et al.*, 1980) and IFs (Laurila *et al.*, 1981). However, removal of the nucleus is followed by the breakdown of the Golgi complexes (Shay *et al.*, 1974; Wise and Larsen, 1976). As we have just seen, this does not impede the binding of lectins such as ConA to the cell surface. Euteneuer and Schliwa (1984) studied the locomotion of

cytoplasts from fish epidermal keratinocytes. Despite the absence of microtubules and centrioles, these cytoplasts are capable, as whole cells, of oriented locomotion.

The reactions of cytoplasts to agents that induce morphological changes in whole cells are also perfectly normal. Among such agents are dibutyryl-cAMP and protaglandin E_1, which affect cell shape in both intact cells and cytoplasts by an MT assembly–dependent process (Schröder and Hsie, 1973). Neuroblastoma cells as well as neuroblastoma cytoplasts respond to the addition of cAMP by neurite formation (Miller and Ruddle, 1974). Finally, viral DNA (obtained from an avian sarcoma virus) replicates in cytoplasts made from fibroblasts (Varmus *et al.*, 1974).

Interestingly, mitoplasts obtained by cytochalasin B treatment of dividing cells behave like mitotic cells; they remain spherical and do not attach to the substratum (Sunkara *et al.*, 1977). Even the tiny microplasts studied by Albrecht-Buehler (1980) retain some of the activities of fibroblasts for some time. These very small pieces of cytoplasm, which die within 8 hr, can form filopodia and membrane ruffles, but are incapable of coordinated locomotion.

One might be tempted to conclude from this discussion that the only function of the nucleus is to allow cell division and reproduction. This is such an essential function that life would soon disappear if all cells lost their chromosomal DNA. However, it would be inappropriate to claim that cytoplasts are equivalent to whole cells in all respects. For instance, Otteskog *et al.* (1981) have shown that cytochalasin B can induce capping of antibodies in transformed (malignant) cells, but not in normal ones. Although cytochalasin B binds to cytoplasts formed from transformed cells, it does not induce capping of antibodies. The conclusion is that either the nucleus or a nucleus-associated organelle controls the mobility of plasma membrane molecules, at least in transformed cells.

It is in their *biochemical properties* that cytoplasts are, in general, inferior to whole cells. In cytoplasts there is no synthesis of nuclear DNA, and RNA synthesis stops very quickly. Although protein synthesis continues, it proceeds at a lower rate than that found in whole cells and its rate decreases continuously (Poste, 1972; Follett, 1974; Shay *et al.*, 1974; Bruno and Lucas, 1983); the rate of both RNA and protein synthesis is very low in mitoplasts (Sunkara *et al.*, 1977). Cholesterol synthesis is not affected for at least 6 hr by removal of the nucleus (Cavenee *et al.*, 1981), but both synthesis and turnover of the coenzyme NAD are modified. According to Rechsteiner and Catanzarite (1974), the enzyme responsible for NAD synthesis (NAD pyrophosphorylase) is present only in the karyoplasts and is thus localized predominantly or entirely in the nucleus. There is no rapid destruction or turnover of NAD in the cytoplasts, whereas turnover of NAD is faster in karyoplasts because the nucleus contains most or all of the poly(ADP) ribose phosphorylase activity; the substrate of this enzyme is NAD (Rechsteiner *et al.*, 1976). Cytoplasts are also deficient in polyamine synthesis. They synthesize putrescine at a normal rate but are unable to convert it

into the spermidine, leading to the conclusion that in fibroblasts ornithine decarboxylase (ODC) is probably ubiquitous in the cell, while *S*-adenosylmethionine-decarboxylase (SAM) is apparently not present in the cytoplasm (McCormick, 1977). In contrast to complete cells, cytoplasts are unable to control amino acid transport properly when the amino acid concentration of the medium is changed experimentally (Hume *et al.*, 1975). It is well known that hepatoma cells respond to the addition of glucocorticoids by the synthesis of tyrosine aminotransferase (TAT). This induced enzyme synthesis is controlled by nuclear genes, since glucocorticoids do not induce TAT activity in the cytoplasts of hepatoma cells (Ivarie *et al.*, 1977). In these cells, enucleation slows the degradation of another enzyme, glutamine synthetase, suggesting the existence of a nuclear control on lysosomal activity (Freidkopf-Cassel and Kulka, 1981). That such a control exists is not very surprising since, as we have seen, removal of the nucleus is quickly followed by disorganization of the Golgi bodies.

If cytoplasts are inferior to complete cells in many respects, the same might be true for the much less studied karyoplasts. Although RNA and proteins are synthesized, this occurs for a few hours only (Ege *et al.*, 1974b; Shay *et al.*, 1974). Karyoplasts are unable to regenerate and divide unless they succeed in attaching to the substratum. The poor macromolecular synthesis in karyoplasts is probably the result of their small size and very abnormal nucleocytoplasmic ratio. A similar condition prevails in the polar bodies expelled during oocyte maturation, which might be responsible for their still unexplained rapid degradation.

Finally, it would be a mistake to believe that the controls exerted by the nucleus on the cytoplasm are always positive. England *et al.* (1978) observed a temporary increase in mitochondrial protein synthesis in cytoplasts. Other examples of this negative nuclear control of mitochondrial activity will be discussed when we deal with eggs (Chapter 2).

B. Reticulocytes

Nature itself performs an enucleation experiment during mammalian erythropoiesis, when nuclei and mitochondria are cast out of differentiating cells. The resulting anucleate reticulocytes contain a basophilic "reticulofilamentous" substance. Electron microscopy has revealed the presence of many polyribosomes in this substance whose main function is the synthesis of a major specific protein, hemoglobin. Synthesis of this protein, coded by the nuclear genes for α- and β-globins, continues for a few days after the nucleus has been expelled. This is possible because the α- and β-globin mRNAs remain functional in the absence of the corresponding nuclear genes. During erythrocyte maturation (Fig. 2), these mRNAs break down and the polyribosomes disintegrate into individual monoribosomes. Finally, the latter also undergo complete degradation, leaving an adult red blood cell (or erythrocyte), which is little more than a bag surrounded by a membrane filled with hemoglobin. Mammalian red blood cells are easily