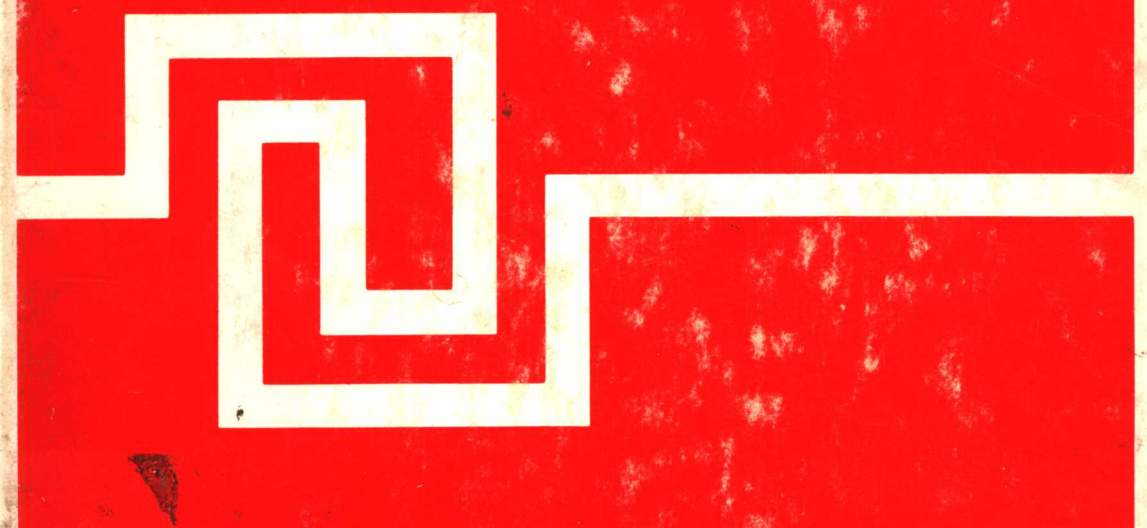
Cell Growth

Edited by
Claudio Nicolini



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Edited by

Claudio Nicolini
Temple University Health Sciences Center
Philadelphia, Pennsylvania
and National Research Council
Genoa, Italy

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PREFACE

During October 18-31, 1980, the first course of the International School of Pure and Applied Biostructure, a NATO Advanced Study Institute was held at the "Ettore Majorana Center for Scientific Culture" in Erice, Sicily, co-sponsored by national and international agencies. The subject of the course was "Cell Growth", with participants (from 16 different countries) selected worldwide.

The study of cell growth has been one of humanity's most challenging problems and it has been approached from many different points of view, such as biochemistry, genetic engineering, cell biology, zoology, oncology, immunology, biophysics and a few other fields. It has been very difficult to keep such varied points of view all in one room and in one audience, because of the heterogeneity of background and inherent difficulty of communication, with occasional nominalistic rather than factual debates. This Institute aimed to bypass those limitations by approaching in a structured and tutorial fashion the problem of cell growth in three dimensions: (1) in terms of the various disciplines involved, from molecular to cellular biology, from genetic engineering to clinical oncology, from biophysics to immunology; (2) in terms of the system studied, from prokaryotes to eukaryotes and cancer cells; (3) in terms of the various levels of macromolecular organization, from membrane to cytoskeleton and chromatin. The emphasis has been placed in the basic sciences, which in the long term constitute the only route to a complete understanding of the molecular-cellular mechanisms regulating growth, differentiation and ultimately cancer.

This book is the result of this Advanced Study Institute and aims to present a structured and widely interdisciplinary view of the current knowledge on normal and abnormal cell growth, up to the very recent findings. Limited space is also left to the presentation of controversial opinions (not necessarily shared by the editor) to permit an unbiased update of the state of the art. The book has been edited in a tutorial format, with the

cooperation of several leading scientists. We wish to express our gratitude to Frank Kendall and Wolfried Linden for their invaluable and critical cooperation prior, during and after the Institute and publication of this volume.

Claudio Nicolini

INTRODUCTION

A Perspective View of the Cell Cycle

Daniel Mazia

Hopkins Marine Station of Stanford University

Pacific Grove, California 93950, U.S.A.

The intensive study of the Cell Cycle has a 30-year history, during which it has grown from an activity of a few cell biologists into an industry. Having learned a good deal by pursuing some rather simple assumptions, the field is ready to replace answers with questions, even to go so far as to ask: "What are the real problems of the Cell Cycle?" One felt this in discussions that filled our days on a mountain-top in ancient Sicily.

The modern history of the field began with and remains dominated by a single discovery, made around 1950. A fact simple in itself - that DNA doubled between cell divisions - gave us the S-phase. It followed from early results that entrance into the S-phase was a commitment to future division. The standardization of the autoradiographic technique, especially after the introduction of tritiated thymidine, defined and conventionalized the phases of the cycle, although they had no inherent content but merely said that in some cells (which became "typical" for no other reason) something happened between the previous division and the beginning of S and something happened between the end of S and the next division. The central problem of the Cell Cycle became the search for the events (so far eluding us) which command or allow the cell to enter S. This emphasis is justified by the best of reasons: it promises a key to the understanding of order or anarchy in the production of cells in higher organisms. However, cell biologists who want to know how one cell becomes two cells have to deal with problems other than the initiation of the replication of DNA and may even find fruitful approaches to the latter problem in considering the cell cycle as a whole.

In the oldest thought on cell reproduction, the greatest emphasis was placed on the Cell Cycle as a sequence of doubling and halving of cell size. The prevailing view was that the completion of the growth of a cell at a "critical mass" was the trigger to cell division. The old idea is easily dismissed by appeal to exceptions but embodies some deep questions of the Cell Cycle: how do we explain the upper limit of cell mass that can be serviced by one nucleus; how can a cell sense its size; what are the special events that take place at the boundary between interphase and mitosis? Current work, especially on yeast cells, reopens the question of a causal relation between cell size and the onset of division. Other work, of which much more is needed, searches for molecules which are synthesized at the end of G2 (when there is a G2 phase).

Now some advances in the rapidly growing field of the structure of chromatin are very promising; we can expect to know the difference between chromatin in interphase and chromatin in mitosis; after all, the condensation of chromatin is the first sign of mitosis. Thus, the early history of the study of the Cell Cycle gave us the two revolutionary events which define the cycle: the initiation of chromosome replication and the initiation of mitosis.

In the discussions at Erice, we were prepared to question the reality of the conventional phases of the Cell Cycle. Considering all of the events by which one cell becomes two, there is no rule that requires that certain ones must take place between division and the beginning of S and others must take place between the end of S and the onset of mitosis. One can purify the paradigm of the Cell Cycle by focussing on what may be called the Reproductive Wheel. In one turn of the Reproductive Wheel, one nucleus makes two equivalent and separated nuclei and the cell is divided. We would say that one cell has made two cells whether or not the cell has grown. We would then see growth in the usual cycle (doubling and halving) as the augmentation of cellular structure and biochemical machinery that supports the turn of the Reproductive Wheel. If a cell has a G1 period, it would only be saying that some events needed for continuing into chromosome replication take place - in that kind of cell - during a period following division. If there is no G1 period, the activities required for starting S took place in the previous generation. If there is no G2, all of the events necessary for entrance into mitosis were completed before the end of S. The events included in cell growth impose the limits on the reproductive events, but they need not follow similar schedules in different kinds of cells.

It is instructive to examine a special kind of cell in which the cell cycle is liberated from restraints of growth: the egg cell. In such cells, it may take as little as 10 minutes to double the number of nuclei. The Reproductive Wheel can turn that rapidly when it is fully supplied with all that it needs. We do not, however, say that such a cycle is independent of events which, in other cells, fall within G1 and G2. On the contrary, we recognize that the maturation of the egg, which may have taken weeks or months, has "fattened" the huge cell with those products which other cells need time to make within a period between divisions, some before the onset of S, others between the end of S and the start of mitosis. What we want to know is the events or molecules which determine the turning of the Reproductive Wheel. The conventional phases of the cycle can help us in working on particular types of cells but will disappoint us if we generalize them.

The Reproductive Wheel expresses itself above all in the Chromosome Cycle. What we can determine easily is that the chromosomes decondense at the time of division; replicate between divisions; begin to condense at the beginning of mitosis; split (separating sister chromosomes) at the end of metaphase; move to the poles and decondense for the next cycle. Some of the chapters in this volume touch on an idea that links the Chromosome Cycle even more closely to the Cell Cycle as a whole. There is evidence that chromosomes decondense gradually (in cells which have a G1) as a cell passes from division into the next interphase. It is possible to speculate that the chromosomes must reach a certain degree of decondensation before they can replicate and that they cannot condense again until they have replicated. If we could consider the Chromosome Cycle to be the foundation of the Cell Cycle, it would provide an intelligible focus for the study of the infinity of events which make up the description of the life history of the cell.

The reader will find in this volume a great wealth of new knowledge about the growing and reproducing cell, viewed at all levels from minutely molecular analysis to the consideration of cell populations. The organizers of the School at Erice aimed beyond the communication of the results of single research efforts toward criticism and judgment. One hopes that the spirit of the School will come through in these pages.

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SECTION I:
WHAT IS A CELL?

STRUCTURE OF THE EUKARYOTIC CELL

Nanne Nanninga

Department of Electron Microscopy and Molecular Cytology

University of Amsterdam, Amsterdam, The Netherlands

INTRODUCTION

The purpose of this tutorial review is to present some recent advances on the structure and origin of the eukaryotic cell. There are many types of eukaryotic cells and, therefore, some abstraction is unavoidable. In addition, nowadays cell biologists have a clear preference for animal cells as compared to plant cells. Within the group of animal cells again a limited number of cell types are being studied. These limitations should be kept in mind.

I have attempted to avoid merely cataloguing a number of cellular structures. When looking back into the past, i.e. the last twenty five years it is possible to find a common denominator. In many instances the picture one has in mind of the eukaryotic cell is largely determined by the electron microscopic technique used. As will be shown below, a technique that allows in particular the visualization of membranes will lead to a cell concept in which the occurrance of membrane-bounded compartments is stressed. By contrast, a technique that preserves the structures inbetween the membranes will lead to emphasis on the non-membranous cellular skeleton. It is within this framework that I would like to present the material.

HISTORICAL ASPECTS

A fascinating period in the study of cells has been the passing of the border between microscopic and submicroscopic structures.

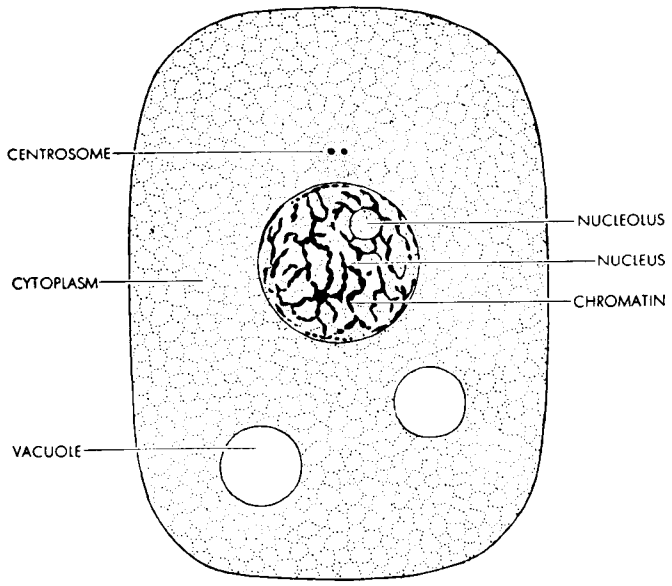

Fig. 1. Cell structure typical for the pre-electron microscopic era (1922). From "The Living Cell" by J. Brachet, Scientific American, September 1961.

This can be clearly seen from the pioneering work of Frey-Wyssling (1, 2). An illustrative example is the evolution (or involution) of a concept like groundplasm: "What is left over after removal of all known particulate bodies of the cytoplasm is a homogeneous mass as seen in the electronmicroscope which we call groundplasm" (2). An old scheme of the cell shows an ill-defined texture between nucleus and cell boundary (Fig. 1). Various investigators have stressed the fibrillar, granular and/or reticular aspect of the groundplasm. Progress was hampered by the limited resolution of the light microscope and the often unindentified occurrance of preparation artefacts. This state of affairs was continued with the emergence of electron microscopy, though at a higher resolution. Typical examples are the following. Bretschneider (3) advocated a detailed reticular concept (Fig. 2a), Frey-Wyssling (1) considered globular macromolecules as elementary units. These would associate (theory of junctions; in German "Hafpunkt-Theorie") to form fibrillar elements, macromolecular films or three-dimensional porous textures of the plasma gel (Fig. 2b). A now strikingly modern concept was obtained by Haguenau and Bernhard (4) in 1952 (Figs. 3a and b).

Improved electron microscopic techniques brought into focus the lamellar aspect of the ground and cytoplasm. Important advances were achieved by Sjöstrand and coworkers (e.g., ref. 5).

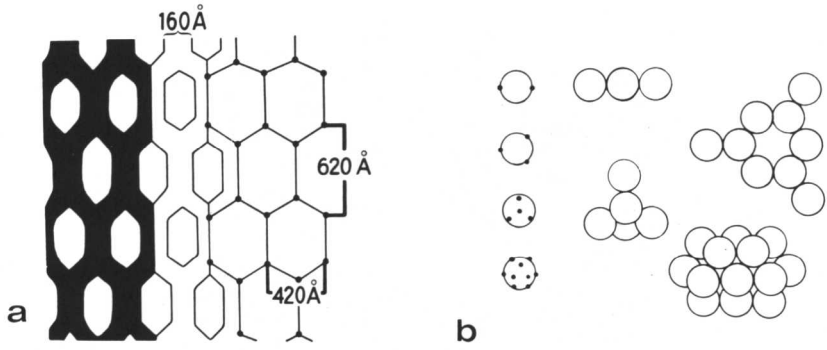

Fig. 2. Hypothetical structure of the groundplasm. (a) detailed proposition of Bretschneider (3) as based on chemical experiments; (b) theory of junctions of Frey-Wyssling (1). Globular macromolecules aggregate by junctions. From top till bottom: coordination number 2, beaded chain; coordination number 3, porous film; coordination number 4, tetrahedral space group; coordination number 12, close packing.

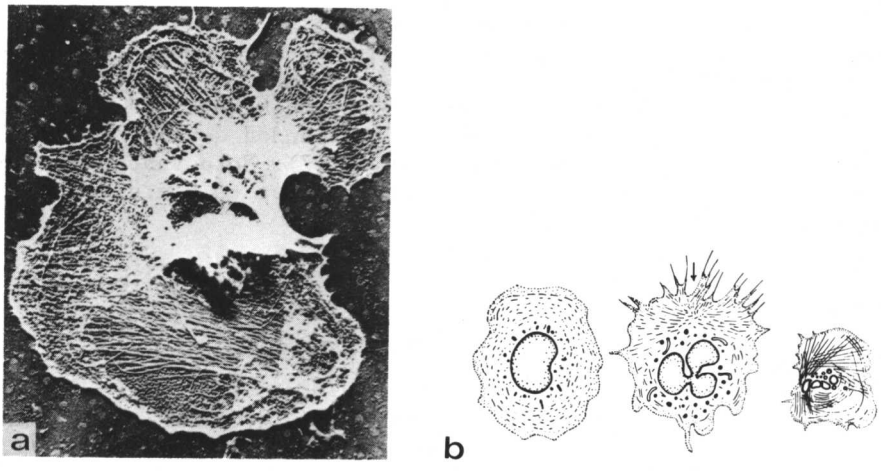

Fig. 3. (a) Fibrillar structure of the groundplasm in a blood platelet (4). Fixation with chromic acid; (b) arrangement of submicroscopic fibrils in (from left to right) a leukocyte, a multinuclear granulocyte and a blood platelet.

The lamellar or membranous aspect of the cell was especially prominent after application of potassium permanganate as a fixative. Membranes became visible as triple-layered structures (unit membrane