Biology and Radiobiology of

ANUCLEATE SYSTEMS

I.

BACTERIA AND ANIMAL CELLS

edited by

Silvano Bonotto **Roland Goutier**

René Kirchmann **Jean-René Maisin**

Biology and Radiobiology of ANUCLEATE SYSTEMS

I. BACTERIA AND ANIMAL CELLS

edited by

Silvano Bonotto Roland Goutier
René Kirchmann Jean-René Maisin

Département de Radiobiologie
Centre d'Etude de l'Energie Nucléaire
Mol, Belgium

Academic Press
New York and London
1972

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

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

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 72-78874

PRINTED IN THE UNITED STATES OF AMERICA

PARTICIPANTS

Henri Alexandre, Département de Biologie Moléculaire, Laboratoire de Cytologie et Embryologie Moléculaires, Université Libre de Bruxelles, Rhode-St. Genèse, Belgium

Christa Anders, Institut für Entwicklungsphysiologie der Universität zu Köln, Cologne, Germany

Zénon M. Bacq, Laboratoire de Physiopathologie, Université de Liège, Liège, Belgium

Cyrille Baes, Département de Radiobiologie, C.E.N./S.C.K., Mol, Belgium

Wilfried Baeyens, Département de Radiobiologie, C.E.N./S.C.K., Mol, Belgium

Corrado Baglioni, Massachusetts Institute of Technology, Cambridge, Massachusetts

Danielle Bailly, Département de Radiobiologie, C.E.N./S.C.K., Mol, Belgium

Hubert Balluet, Département de Radiobiologie, C.E.N./S.C.K., Mol, Belgium

Lucille Baugnet-Mahieu, Département de Radiobiologie, C.E.N./S.C.K., Mol, Belgium

Sigrid Berger, Max-Planck-Institut für Zellbiologie, Wilhelmshaven, Germany

Monique Boloukhère, Département de Biologie Moléculaire, Laboratoire de Cytologie et Embryologie Moléculaires, Université Libre de Bruxelles, Rhode-St. Genèse, Belgium

Eliane Bonnijns-Van Gelder, Département de Radiobiologie, C.E.N./S.C.K., Mol, Belgium

Silvano Bonotto, Département de Radiobiologie, C.E.N./S.C.K., Mol, Belgium

Jean Brachet, Département de Biologie Moléculaire, Laboratoire de Cytologie et Embryologie Moléculaires, Université Libre de Bruxelles, Rhode-St. Genèse, Belgium

Ernst P. O. Brändle, Institut für Biologie der Universität Tübingen, Tübingen, Germany

Horst Bresch, Medizinische Hochschule Hannover, Abteilung für experimentelle Pathologie, Theoretische Institute I, Hanover, Germany

Marc Callebaut, Laboratorium voor Anatomie en Embryologie, Rijksuniversitair Centrum, Fakulteit der Wetenschappen, Antwerp, Belgium

Pol Charles, Département de Radiobiologie, C.E.N./S.C.K., Mol, Belgium

Sydney P. Craig, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland

Anne Catherine Dazy, Institut de Biologie Cellulaire Végétale, Université de Paris, Paris, France

Alain Declève, Département de Radiobiologie, C.E.N./S.C.K., Mol, Belgium

Ghislain Deknudt, Département de Radiobiologie, C.E.N./S.C.K., Mol, Belgium

Thomas D'Souza, Biology Division, Bhabha Atomic Research Centre, Trombay, Bombay, India

R. E. Ecker, Biological and Medical Research Division, Argonne National Laboratory, Argonne, Illinois

Maurice Errera, Laboratoire de Biophysique et Radiobiologie, Université Libre de Bruxelles, Rhode-St. Genèse, Belgium

Eugène Fagniart, Département de Radiobiologie, C.E.N./S.C.K., Mol, Belgium

Henri Firket, Institut de Pathologie, Université de Liège, Liège, Belgium

Georg Gerber, Département de Radiobiologie, C.E.N./S.C.K., Mol, Belgium

Yves Gérin, Département de Biologie Moléculaire, Laboratoire de Cytologie et Embryologie Moléculaires, Université Libre de Bruxelles, Rhode-St. Genèse, Belgium

Norberto Gilliavod, Département de Radiobiologie, C.E.N./S.C.K., Mol, Belgium

Antonio Giuditta, International Institute of Genetics and Biophysics, Naples, Italy

André Goffeau, EURATOM, Université de Louvain, Heverlee, Belgium

Roland Goutier, Département de Radiobiologie, C.E.N./S.C.K., Mol, Belgium

Johannes Hackstein, Zoologisches Institut, Lehrstuhl: Experimentelle Morphologie, Universität zu Köln, Cologne, Germany

Francoise Andrée Hanocq, Département de Biologie Moléculaire, Laboratoire de Cytologie et Embryologie Moléculaires, Université Libre de Bruxelles, Rhode-St. Genèse, Belgium

Maurice N. Harford, Département de Radiobiologie, C.E.N./S.C.K., Mol, Belgium

Raoul Huart, Département de Radiobiologie, C.E.N./S.C.K., Mol, Belgium

Paavo Kallio, Department of Botany, University of Turku, Finland

Fernand Kennes, Département de Radiobiologie, C.E.N./S.C.K., Mol, Belgium

Micheline Kirch, Département de Biologie Moléculaire, Laboratoire de Cytologie et Embryologie Moléculaires, Université Libre de Bruxelles, Rhode-St. Genèse, Belgium

René Kirchmann, Département de Radiobiologie, C.E.N./S.C.K., Mol, Belgium

Klaus Kloppstech, Max-Planck-Institut für Zellbiologie, Wilhelmshaven, Germany

Luc Lateur, Département de Biologie Moléculaire, Laboratoire de Cytologie et Embryologie Moléculaires, Université Libre de Bruxelles, Rhode-St. Genèse, Belgium

Lucien Ledoux, Département de Radiobiologie, C.E.N./S.C.K., Mol, Belgium

Rachel M. Leech, Department of Biology, University of York, Heslington, England

Mark Lemarcq, Département de Biologie Moléculaire, Laboratoire de Cytologie et Embryologie Moléculaires, Université Libre de Bruxelles, Rhode-St. Genèse, Belgium

Alain Léonard, Département de Radiobiologie, C.E.N./S.C.K., Mol, Belgium

Gerno Linden, Département de Radiobiologie, C.E.N./S.C.K., Mol, Belgium

Paul Lurquin, Département de Radiobiologie, C.E.N./S.C.K., Mol, Belgium

Jean-René Maisin, Département de Radiobiologie, C.E.N./S.C.K., Mol, Belgium

Paul Manil, Faculté des Sciences Agronomiques, Gembloux, Belgium

G. Marbaix, Department of Zoology, University of Oxford, England

Max Mergeay, Département de Radiobiologie, C.E.N./S.C.K., Mol, Belgium

Catherine Micholet-Côté, Département de Radiobiologie, C.E.N./S.C.K., Mol, Belgium

Thomas Palayoor, Cancer Research Institute, Parel, Bombay, India

Evelyne Paulet, Université Libre de Bruxelles, Rhode-St. Genèse, Belgium

Simone Puiseux-Dao, Institut de Biologie Cellulaire Végétale, Université de Paris, Paris, France

Jacques Remy, Département de Radiobiologie, C.E.N./S.C.K., Mol, Belgium

Matthieu Ricard, Institut Pasteur, Paris, France

Gerhard Richter, Institut für Botanik der Technischen Universität Hannover, Hanover, Germany

André Sassen, Département de Radiobiologie, C.E.N./S.C.K., Mol, Belgium

J. F. Scaife, Biology Division, EURATOM, Ispra, Italy

G. G. Selman, Institute of Animal Genetics, University of Edinburgh, Scotland

Akihiro Shima, Laboratory of Radiation Biology, Faculty of Science, University of Tokyo, Japan

Yvette Škreb, Institute for Medical Research, Yugoslav Academy of Sciences and Arts, Zagreb, Yugoslavia

Julia Swinnen-Vranckx, Département de Radiobiologie, C.E.N./S.C.K., Mol, Belgium

Christian Thomas, Département de Biologie Moléculaire, Laboratoire de Cytologie et Embryologie Moléculaires, Université Libre de Bruxelles, Rhode-St. Genèse, Belgium

René Thomas, Département de Biologie Moléculaire, Laboratoire de Génétique, Université Libre de Bruxelles, Rhode-St. Genèse, Belgium

Monique Tourte, Cytologie et Cytophysiologie de la Photosynthèse, Gif-sur-Yvette, France

Yves Tourte, Laboratoire de Biologie Végétale V, Paris, France

Lucien Vakaet, Laboratorium voor Anatomie en Embryologie, Rijksuniversitair Centrum, Fakulteit der Wetenschappen, Antwerp, Belgium

Thérèse Vanden Driessche, Département de Biologie Moléculaire, Laboratoire de Cytologie et Embryologie Moléculaires, Université Libre de Bruxelles, Rhode-St. Genèse, Belgium

Colette van der Ben, Institut Royal de Sciences Naturelles de Belgique, Brussels, Belgium

Dick van der Ben, Institut Royal de Sciences Naturelles de Belgique, Brussels, Belgium

Oscar Van der Borght, Département de Radiobiologie, C.E.N./S.C.K., Mol, Belgium

Constantin Vilău, Oncologic Institute, Bucharest, Rumania

Harry Waris, University of Helsinki, Helsinki, Finland

Claude Watters, Département de Radiobiologie, C.E.N./S.C.K., Mol, Belgium

C. L. F. Woodcock, Harvard University, The Biological Laboratories, Cambridge, Massachusetts

Yoshio Yoshida, Department of Biology, Faculty of Science, Niigata University, Niigata, Japan

Klaus Zetsche, Institut für Biologie der Universität Tübingen, Tübingen, Germany

PREFACE

During the First International Symposium on *Acetabularia,* held in Brussels and in Mol, June 1969, it became apparent that a comparison of the properties of anucleate *Acetabularia* with those of other anucleate cells would not only be very interesting but very useful for a better understanding of many problems concerning the nucleocytoplasmic relationships and cellular differentiation in normal and irradiated cells. Therefore, we decided to bring together, for the first time, scientists working on anucleate systems obtained from bacteria and animal or plant cells.

A three-day symposium on Biology and Radiobiology of Anucleate Systems was organized in the Department of Radiobiology of the Centre d'Etude de l'Energie Nucléaire (C.E.N./S.C.K.) at Mol, Belgium, June 21-23, 1971, thanks to the generous sponsorship of the Commission of the European Communities (EURATOM), the Relations Culturelles Internationales (Brussels), and the C.E.N./S.C.K. The opening addresses were delivered by Mr. M. Mees, representing Mr. J. Goens, General Director of the C.E.N./S.C.K. We thank Professors Z. M. Bacq, J. Brachet, H. Chantrenne, M. Chevremont, M. Errera, H. Firket, P. Manil, and R. Thomas, who contributed, as Scientific Advisers, to the success of the symposium. We are particularly indebted to Mr. E. Brons, Public Relations Officer, and to all the other members of C.E.N./S.C.K. who helped in some way in the organization of the symposium.

The papers presented at the symposium generally concern anucleate systems; a few papers, however, deal with some very real problems (function of membrane-bound polyribosomes, behavior of isolated cellular organelles) of interest to the investigator of anucleate systems.

We hope that these proceedings, published in two volumes, will contribute in some way to a better knowledge of the normal and irradiated cell and of the subtle relations between its nucleus and cytoplasm. Volume I is comprised of an opening lecture delivered by Professor J. Brachet and eleven papers on bacteria and animal cells. Volume II is comprised of sixteen papers relating to plant cells.

Silvano Bonotto
Roland Goutier
René Kirchmann
Jean-René Maisin

CONTENTS

SESSION 3

Chairman: Maurice Errera

JUNE 22

SESSION 4

Chairman: Lucien Vakaet

MORPHOGENESIS AND SYNTHESIS OF MACROMOLECULES IN THE ABSENCE OF THE NUCLEUS[x]

Jean Brachet

Laboratory of Molecular Cytology and Embryology
Department of Molecular Biology
University of Brussels (Belgium)

and

Laboratory of Molecular Embryology
Consiglio nazionale delle Ricerche (Italy)

Introduction

The synthesis of macromolecules such as DNA, RNA and proteins is the result of complex nucleocytoplasmic interactions, which can be studied in a number of ways : biochemical analysis of isolated cell organelles (nuclei, mitochondria, microsomes, etc...), autoradiography (which can be performed on intact cells), comparison of nucleate and anucleate fragments of unicellular organisms or eggs. All these approaches have their advantages and their drawbacks. Biochemical studies afford the best opportunities for a detailed analysis of synthetic processes ; but one is never sure, when one is working with isolated nuclei, for instance, that certain molecules have not leaked out of the nuclei or that cytoplasmic components have not been adsorbed unspecifically on their surface : cytoplasmic contamination of isolated nuclei is often difficult to rule out, while the true intracellular localization of enzymes found

[x]Opening lecture of the Symposium

in the " cell sap" often remains open to question. Autoradiography, which can now be used at the electron microscope level without difficulty, gives exceedingly valuable information about the intracellular sites of macromolecular synthesis; but it cannot be easily used for the study of the size of the precursor pool (free amino acids, nucleotides, etc...), so that the specific activity of the neosynthesized macromolecules usually remains unknown. Comparative study of nucleate and anucleate fragments of cells, which is the topic of the present Symposium, would be ideal if the amount of material, which can be obtained by microsurgery, was not often so small that delicate and time-consuming ultra-micromethods have to be used for biochemical analysis. Exceptions to this statement are the alga Acetabularia because of its large size, and the sea-urchin eggs, which can be cut into two halves by centrifugation : this is why we know much more about the biochemistry of anucleate Acetabularia and sea-urchin eggs than about that of Stentor or Micrasterias.

It is clear that, if we wish to understand better nucleocytoplasmic interactions in morphogenesis and synthesis of macromolecules, all available methods should be used : this is why, in the present introduction, the work done on anucleate systems will be integrated in the broader frame of the nucleocytoplasmic interactions in morphogenesis.

Before going into the subject, it is worth pointing out that the three above-mentioned approaches (biochemical analysis of isolated cell organelles, autoradiography and studies on anucleate systems) have led to the same general conclusions : the main function of the nucleus is not, as was believed in the past, energy production or protein synthesis; it is the synthesis of nucleic acids, i.e. DNA and all kinds of RNA's.

In the following, we shall first discuss our present ideas on nucleocytoplasmic interactions in cell differentiation (in embryos, particularly), then examine some of the facts on which these concepts have been based.

Theories of differentiation

Many classical experiments have clearly established the importance of cytoplasmic organization during the very early stages of embryonic development : for instance, removal of the " polar lobe" at the trefoil stage of cleavage results, in molluscs, in various defects in the larva; mild centrifugation of a fertilized amphibian egg induces marked microcephaly, while inversion of its polarity, by keeping the egg upside down produces siamese twins. The dorsoventral organization of the amphibian egg can be established at will by the experimenter by simply forcing the egg to rotate in a given plane. As a result of this rotation, the dorsal side becomes visible by the appearance of the so called grey crescent. If the cortex (a few µm thick) of this dorsal side is removed, the egg will cleave and form a blastula, but it will not develop further. If, on the contrary, the dorsal cortex of a fertilized egg is grafted on the ventral side of another fertilized egg, a double embryo will form (Curtis, 1960). In all these experiments, nothing is apparently done to the egg nucleus, while minor alterations of the cytoplasm have far-reaching consequences for further development.

On the other hand, many experiments in which the nucleus has been intentionally injured (in order to produce mutations, aneuploidy, haploidy, hybridization, etc...) definitely show that nuclear integrity is required in order to obtain full and normal development. This effect of the nucleus is hardly conspicuous until the initial period of cleavage is over. But morphogenetic movements, neural induction, organogenesis, tissue and cell differentiation all require the presence of normal, genetically active, nuclei.

Morgan (1934), who was not only an outstanding geneticist, but also a very distinguished experimental embryologist, tried, in 1934, to present a coherent theory of nucleocytoplasmic interactions during development. His basic idea was that the cytoplasmic organization of the egg being heterogeneous, the nuclei (which would all contain the same genes) will be distributed, at the end of cleavage, in regions of the blastula which are chemically different from each other. As a result, genes would become " activated" in certain parts of the embryo and not in other areas. Under

the impulse of the activated genes, the cytoplasm would become still more different in the various parts of the embryo : this would lead to further gene activation, and so forth, until the cells have differentiated.

This theory perhaps remains the best one we have when we try to explain embryonic differentiation : in its modern form, it will be said that genes were repressed during cleavage and that they undergo selective derepression during development. Differentiation would result from differential gene activation ; this means that the genes which direct the synthesis of a given protein (haemoglobin, for instance) will be activated (or derepressed) in a given area at a given stage of development. But what causes genes to be " switched" on and off, in a very specific way, remains the great mystery of cell differentiation.

Other theories have been proposed, in recent days, in order to explain cell differentiation : Jacob & Monod (1963) tried to extend their model for gene regulation in bacteria to the differentiating cell. A much more elaborate model was proposed, in 1969, by Britten & Davidson (1969) ; in addition to the fact that the complexity of the model makes it difficult to test experimentally, it suffers from the drawback that it does not take into account cytoplasmic heterogeneity. The lack of interest for possible cytoplasmic controls of gene activity is rather surprising, since Davidson, Allfrey & Mirsky (1964) showed that, in the mollusc Ilyanassa, removal of the polar lobe (a purely cytoplasmic area) affects in a negative way RNA synthesis in the "lobeless" embryo : gene activation is still repressed many hours after the surgical removal of the polar lobe.

Scarano and his associates (1967, 1969) have suggested that specific methylation of DNA might have a determining effect on development. Their experimental findings, though interesting, are not yet quite conclusive. But a major objection to any theory based on chemical modifications of the DNA molecules themselves lies in the fact, which will be discussed later, that the nuclei of the adult still contain, in an active form, all the genes needed for complete differentiation (review by Gurdon & Woodland, 1970). Furthermore, there are many well demonstrated cases of cell dedifferentiation, obtained by simple modifications of the "in vitro" culture conditions.

A new and tempting theory of cell differentiation is that of selective gene amplification : the genes which code for the synthesis of a tissue specific protein (haemoglobin for instance) would undergo selective replication in the cells which will produce that protein. It is well known that enormous (over 1000 times) gene amplification occurs in the case of the ribosomal RNA's (r-RNA's) genes during the ovogenesis of the amphibians : the result is a tremendous synthesis of ribosomes during this period. It is less known that Ficq & Pavan have shown, in 1957, that individual genes (which are visible under the microscope in the giant chromosomes of the insect Rhynchosciara) undergo selective replication during larval life. Whether selective gene amplification can account for cell differentiation remains to be seen, but the possibility must be considered very seriously.

Other, less popular, theories shift the emphasis from the nuclear genes to the cytoplasm : they derive, in one way or the other, from the once fashionable plasmagene hypothesis. The main assumption is the presence, in the cytoplasm, of self-reproducible, specific particles (the plasmagenes). An egg would contain a complex population of plasmagenes and differentiation would be the result of a competition between them in the various embryonic areas.

There is no doubt that many of the plasmagenes which were studied some 25 years ago are nothing but viruses. We are now left with very few cytoplasmic organelles which can self-reproduce : the centrosomes of the dividing cell, the kinetosomes of the cilia and flagella, the mitochondria, and the chloroplasts of green plant cells. Since both mitochondria and chloroplasts contain DNA and can be the site of cytoplasmic, non-mendelian mutations, they might theoretically play a role in cell differentiation. In certain invertebrate eggs, of the so called "mosaic" type, unequal distribution of mitochondria is a well-established fact (Reverberi, 1961) : for instance, in Tunicates, there seems to exist a close correlation between an accumulation of mitochondria in certain parts of the egg and the differentiation of the muscles in the tadpole larva. Although this correlation still holds true in centrifuged eggs, it would be unrealistic to explain the facts on the basis of a plasmagene theory. It seems more likely and simpler to as-

sume that muscle differentiation, in an ascidian tadpole, requires a large amount of energy, which would be provided by the mitochondria.

Recently, the plasmagene theory has been reviewed by Bell (1969) and by Curtis (1967). Bell claims the existence in the cytoplasm of "in vitro" cultivated embryonic cells, of DNA-containing particles which he has called, I-somes (I standing for information). The I-somes would be made of "informational" DNA and proteins ; they would, like the plasmagenes, carry genetic information and be capable of self-replication in the cytoplasm. But the reality of the I-somes is very much in doubt now : it is probable that they are just artefacts originating from broken or degenerating nuclei.

The work of Curtis (1965) is of an entirely different kind, since it is based on genetic and not on biochemical considerations. Curtis (1965) observed that, after light injury of the grey crescent in the toad Xenopus, some of the eggs never gastrulate and die as late blastulae. But others develop perfectly and become adults which yield eggs and sperm. They can thus be crossed with normal toads. A cross between a male originating from an egg whose grey crescent (dorsal cortex) had been injured and a normal female gives normal descendance. But the reciprocal cross (between a female originating from an egg whose grey crescent had been injured and a normal male) gives a high proportion of lethals : they die, as if their own grey crescent had been removed, at the late blastula or early gastrula stage. This means that the dorsal cortex apparently has its own heredity : such a "cortical heredity" implies that it must contain self replicating cytoplasmic particles comparable to the plasmagenes. This hypothesis is, in fact, an extension to the fertilized amphibian egg of the ideas of Sonneborn (recent review in 1970), who has produced very strong evidence for the existence of a cortical heredity in Paramecium.