Academic Press Rapid Manuscript Reproduction

Proceedings of the 1979 ICN–UCLA Symposia on
Molecular and Cellular Biology held in Keystone,
Colorado, March 11–16, 1979

ICN–UCLA Symposia on Molecular and Cellular Biology
Volume XV, 1979

EXTRACHROMOSOMAL DNA

edited by

DONALD J. CUMMINGS
Department of Microbiology and Immunology
University of Colorado Medical Center
Denver, Colorado

PIET BORST
Jan Swammerdam Institute
University of Amsterdam
Amsterdam, Netherlands

IGOR B. DAWID
Laboratory of Biochemistry
National Cancer Institute
National Institutes of Health
Bethesda, Maryland

SHERMAN M. WEISSMAN
School of Medicine
Yale University
New Haven, Connecticut

C. FRED FOX
Department of Bacteriology and Molecular Biology Institute
University of California, Los Angeles
Los Angeles, California

ACADEMIC PRESS 1979
A Subsidiary of Harcourt Brace Jovanovich, Publishers
New York London Toronto Sydney San Francisco

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 7DX

ISBN 0-12-198780-9

PRINTED IN THE UNITED STATES OF AMERICA

79 80 81 82 9 8 7 6 5 4 3 2 1

CONTRIBUTORS

Numbers in parentheses refer to chapter numbers

ALZNER-DEWEERD B. (24), Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

ARNBERG, A. C. (20), Biochemistry Laboratories, University of Groningen, Zernikelaan, Groningen, Netherlands

ASTIN, ANNE (17), Department of Biochemistry, Monash University, Clayton, Victoria 3168 Australia

ATTARDI, GIUSEPPE (28), Division of Biology, California Institute of Technology, Pasadena, California 91125

BATTEY, JIM (27), Department of Pathology, Stanford Medical Center, Stanford California 94305

BEALE, G. H. (1), Institute of Animal Genetics, West Mains Road, Edinburgh, EH9 3JN, Scotland

BELCOUR, L. (34), Centre de Genetique Moleculaire, CNRS, Gif sur Yvette, France

BOER, P. H. (20), University of Amsterdam, Box 60.000, 1005 GA, Amsterdam, Netherlands

BOGORAD, LAWRENCE (8), Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138

BORST, P. (20, 32), University of Amsterdam, Box 60.000, 1005 GA, Amsterdam, Netherlands

BOS, J. L. (20), University of Amsterdam, Box 60.000, 1005 GA, Amsterdam, Netherlands

BOYNTON, J. E. (6), Department of Botany, Duke University, Durham, North Carolina 27706

BROWN, WESLEY M. (30), Department of Biochemistry, University of California, Berkeley, California 94720

BUKHARI, AHMAD, I. (10), Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724

BUTOW, RONALD A. (18), University of Texas, Health Science Center at Dallas, Dallas, Texas 75235

CANTATORE, P. (28), Division of Biology, California Institute of Technology, Pasadena, California 91125

CHACONAS, G. (10), Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724

CHALLBERG, MARK D. (12), Department of Microbiology, 725 North Wolfe Street, Baltimore, Maryland 21205

CHING, E. (28), Division of Biology, California Institute of Technology, Pasadena, California 91125

CHING, HO (31), Laboratory of Cell Biology, National Cancer Institute, NIH, Bethesda, Maryland 20205

CLAYTON, DAVID A. (27), Department of Pathology, Stanford Medical Center, Stanford, California 94305

COON, HAYDEN G. (31), Laboratory of Cell Biology, National Cancer Institute, NIH, Bethesda, Maryland 20205

CREWS, S. (28), Division of Biology, California Institute of Technology, Pasadena, California 91125

CUMMINGS, DONALD J. (3, 34), Department of Microbiology and Immunology, University of Colorado Medical Center, Denver, Colorado 80262

DAWID, I. B. (25), Laboratory of Biochemistry, National Cancer Institute, NIH, Bethesda, Maryland 20205

DE WAARD, ADRIAN (15), Sylvius Laboratories, Physiological Chemistry, 2333, AL Leiden, Netherlands

DIN, N. (4), University of Copenhagen, DK-2200, Copenhagen, Denmark

DONELSON, JOHN E. (23), Department of Biochemistry, University of Iowa, Iowa City, Iowa 52240

DROUIN, JACQUES (29), Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143

DUBOW, M. (10), Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724

EDWARDS. J. (19), Department of Medicine, University of Chicago, Chicago, Illinois 60637

ENGBERG, J. (4), Biochemical Institute, University of Copenhagen, DK-2200, Copenhagen, Denmark

FAURON CHRISTIANE, M.-R. (26), Department of Biology, University of Utah, Salt Lake City, Utah 84112

FAUST, EMANUEL A. (13), Department of Human Genetics, Yale University, New Haven, Connecticut 06510

GELFAND, R. (28), Division of Biology, California Institute of Technology, Pasadena, California 91125

GHOSH, PRABAT K. (14), Department of Human Genetics, Yale University School of Medicine, New Haven, Connecticut 06510

GILLHAM, NICHOLAS W. (6), Department of Zoology, Duke University, Durham, North Carolina 27706

GODDARD, JUDY M. (26), Department of Biology, University of Utah, Salt Lake City, Utah 84112

GOODMAN, HOWARD M. (30), Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143

GRANDCHAMP, C. (34), Centre de Genetique Moleculaire, CNRS, Gif sur Yvette, France

GRANT, D. M. (6), Department of Zoology, Duke University, Durham, North Carolina 27706

GRAY, PATRICK W. (9), Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143

GRINDLEY, NIGEL D. F. (11), Department of Biological Science, University of Pittsburgh, Pittsburgh, Pennsylvania 15260

GRIVELL, LESLIE A. (20), Laboratory of Biochemistry, University of Amsterdam, 1005 GA, Amsterdam, Netherlands

GROOT, G. S. P. (20), Laboratory of Biochemistry, University of Amsterdam, 1005 GA, Amsterdam, Netherlands

HALLICK, RICHARD B. (9), Department of Chemistry, University of Colorado, Boulder, Colorado 80309

HARTLEY, JAMES L. (23), Department of Biochemistry, University of Iowa, Iowa City, Iowa

HECHT, N. B. (20), University of Amsterdam, Box 60.000, 1005 GA, Amsterdam, Netherlands

HECKMAN, J. E. (24), Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

HENSGENS, L. A. M. (20), University of Amsterdam, Box 60.000, 1005 GA, Amsterdam, Netherlands

HOEIJMAKERS, J. H. J. (32), Jan Swammerdam Institute, 1005 GA, Amsterdam, Netherlands

HOLLENBERG, CORNELIS P. (21), Max Planck Institut, Spemannstr. 34, 7400 Tubingen, FRG

HU, W. W. L. (5), Crop Science, North Carolina State University, Raleigh, North Carolina 27650

JOCHEMSEN, HENK (15), Sylvius Laboratories, Physiological Chemistry, 2333 AL Leiden, Netherlands

JOLLY, SETSUKO O. (8), Harvard University, Cambridge, Massachusetts 02138

KELLY, JR., THOMAS J. (12), Department of Microbiology, 725 North Wolfe Street, Baltimore, Maryland 21205

KHATOON, H. (10), Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724

KIDANE, G. (33), Biology Department, University of California, Los Angeles, California 90024

LEBOWITZ, PAUL (14), Department of Human Genetics, Yale University School of Medicine, New Haven, Connecticut 06510

LEVENS, D. (19), Department of Medicine, University of Chicago, Chicago, Illinois 60637

LEVINGS III, C. S. (5), Department of Genetics, North Carolina State University, Raleigh, North Carolina 27650

LI, MAY (22), Department of Biological Sciences, Columbia University, New York, New York 10027

LINK, GERHARD (8), Harvard University, Cambridge, Massachusetts 02138

LINNANE, ANTHONY W. (17), Department of Biochemistry, Monash University, Clayton, Victoria, 3168. Australia

LOCKER, J. (19), University of Chicago, Department of Medicine, Chicago, Illinois 60637

LUPKER, JAN H. (15), Sylvius Laboratories, Physiological Chemistry, 2333 AL Leiden, Netherlands

LUSTIG, A. (19), Department of Medicine, University of Chicago, Chicago, Illinois 60637

MAAT, JAN (15), Sylvius Laboratories, Physiological Chemistry, 2333 AL Leiden, Netherlands

MACINO, GIUSEPPE (22), Instituto di Fisiologia Generale, Universita degli Studi di Roma, Roma, Italy

McINTOSH, LEE (8), Harvard University, Cambridge, Massachussetts 02138

MAHLER, HENRY R. (2), Department of Chemistry, Indiana University, Bloomington, Indiana 47405

MAKI, R. A. (3), University of Colorado Medical Center, Denver Colorado 80262

MARTIN, NANCY C. (23), Department of Biochemistry, University of Minnesota, Minneapolis, Minnesota 55455

MARZUKI, SANGKOT (17), Department of Biochemistry, Monash University, Clayton, Victoria 3168. Australia

MASUDA, H. (33), University of Rio de Janeiro, Cidade Universitaria 20.000, Rio de Janeiro, Brazil

MERKEL, C. (28), Division of Biology, California Institute of Technology, Pasadena, Calfornia 91125

MERTEN, S. (19), Department of Medicine, University of Chicago, Chicago, Illinois 60637

MICHAEL, N. (33), Biology Department, University of California, Los Angeles, California 90024

MILLER, DENNIS L. (23), Department of Biology, University of Iowa, Iowa City, Iowa

MORIMOTO, R. (19), Department of Medicine, University of Chicago, Chicago, Illinois 60637

MOYNIHAN, PATRICK S. (23), Department of Biochemistry, University of Iowa, Iowa City, Iowa

MURPHY, MARK (17), Department of Biochemistry, Monash University, Clayton, Victoria, 3168. Australia

NOBREGA, MARINA P. (22), Department of Biological Sciences, Columbia University, New York, New York 10027

OJALA, D. (28), Division of Biology, California Institute of Technology, Pasadena, California 91125

OROZCO, JR., EMIL M. (9), University of Colorado, Box 215, Boulder, Colorado 80309

PAGANO, JOSEPH S. (16), Cancer Research Center, University of North Carolina, Chapel Hill, North Carolina 27514

PERLMAN, PHILIP S. (2), Department of Genetics, Ohio State University, Columbus, Ohio 43210

PHAM, HUNG DINH (23), Department of Biochemistry, University of Minnesota, Minneapolis, Minnesota

PIATAK, MICHAEL (14), Department of Human Genetics, Yale University School of Medicine, New Haven, Connecticut 06510

PRING, D. R. (5), Department of Plant Pathology, University of Florida, Gainesville, Florida 32611

PRITCHARD, A. E. (3), University of Colorado Medical Center, Denver, Colorado 80262

RABINOWITZ, MURRAY (19), Department of Medicine, University of Chicago, Chicago, Illinois 60637

RAJBHANDARY, U. L. (24), Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

RASTL, E. (25), National Cancer Institute, NIH, Bethesda, Maryland 20205

REDDY, V. BHASKARA (14), Department of Human Genetics, Yale University School of Medicine, New Haven, Connecticut 06510

ROSENBLATT, H. (33), Biology Department, University of California, Los Angeles, California 90024

RUBENSTEIN, JOHN L. R. (27), Department of Pathology, Stanford Medical Center, Stanford, California 94305

RUSHLOW, KEITH E. (9), University of Colorado, Box 215, Boulder, Colorado 80309

SAGER, RUTH (7), Sidney Farber Cancer Institute, Boston, Massachusetts 02115

SCHRIER, PETER I. (15), Sylvius Laboratories, Physiological Chemistry, 2333 AL Leiden, Netherlands

SHAH, D. M. (5), Department of Microbiology, University of Chicago, Chicago, Illinois 60637

SHEPHERD, H. S. (6), Department of Biology, University of California, La Jolla, California 92093

SIGURDSON, CHRIS (23), Department of Biochemistry, University of Minnesota, Minneapolis, Minnesota

SIMPSON, A. M. (33), Biology Department, University of California, Los Angeles, California 90024

SIMPSON, LARRY (33), Biology Department, University of California, Los Angeles, California 90024

SMITH, STUART C. (17), Department of Biochemistry, Monash University, Clayton, Victoria, 3168. Australia

STIEGLER, GARY L. (9), University of Colorado, Box 215, Boulder, Colorado 80309

STRAUSBERG, R. L. (18), University of Texas, Dallas, Texas 75235

SYMONS, ROBERT H. (29), Department of Biochemistry, University of Adelaide, Adelaide, South Australia 5001

SYNENKI, R. (19), Department of Medicine, University of Chicago, Chicago, Illinois 60637

TABAK, H. F. (20), University of Amsterdam, Box 60.000, 1005 GA, Amsterdam, Netherlands

TERPSTRA, P. (18), University of Texas, Dallas, Texas 75235

TIMOTHY, D. H. (5), Crop Science, North Carolina State University, Raleigh, North Carolina 27650

TZAGOLOFF, ALEXANDER (22), Department of Biological Sciences, Columbia University,

VAN BEVEREN, CHARLES P. (15), Sylvius Laboratories, Physiological Chemistry, 2333 AL Leiden, Netherlands

VAN BRUGGEN, E. F. J. (20), Biochemistry Laboratories, University of Gronigen, Zernikelaan, Groningen, Netherlands

VAN DEN ELSEN, PETER J. (15), Sylvius Laboratories, Physiological Chemistry, 2333 AL Leiden, Netherlands

VAN DER EB, ALEX J. (15), Sylvius Laboratories, Physiological Chemistry, 2333 AL Leiden, Netherlands

VAN OMMEN, G. J. B. (20), University of Amsterdam, Box 60.000, 1005 GA Amsterdam, Netherlands

VAN ORMONDT, HANS (15), Sylvius Laboratories, Physiological Chemistry, 2333 AL Leiden, Netherlands

WARD, DAVID C. (13), Department of Human Genetics, Yale University, New Haven, Connecticut 06510

WEISSMAN, SHERMAN M. (14), Department of Human Gentics, Yale University School of Medicine, New Haven, Connecticut 06510

WOLSTENHOLME, DAVID R. (26), Department of Biology, University of Utah, Salt Lake City, Utah 84112

WURTZ, E. A. (6), Department of Physiology and Biophysics, University of Illinois, Urbana, Illinois 61801

YIN, S. (24), Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

PREFACE

In some respects, the organization and fundamental concept of this symposium on extrachromosomal DNA resembles the circular nature of some of the extrachromosomal elements themselves. It is difficult to separate the beginning from the end. The symposium was organized on the premise that the diversity and complexity of primitive mitochondrial and perhaps chloroplast DNA structure and replication had more in common with many viral systems than with either prokaryotic or eukaryotic systems. This is especially striking in the case of so-called split genes. Intervening sequences in DNA were first discovered in the small DNA viruses and later in nuclear genes. But it is in yeast mitochondrial DNA that the extent of their involvement in RNA processing is most noteworthy. As reported at this symposium, it is possible to isolate mutants in some intervening sequences and analyze their effect in loci of known genetic function. Not only will such analyses in yeast mitochondrial split genes lead to a basic understanding of intervening sequences in general, but their very presence will have to be dealt with in evaluating theories on mitochondrial evolution.

It should not be surprising that the most active area of research represented at this meeting is the biogenesis of yeast mitochondria. At the close of the symposium, Pyotr Slonimski presented a brief overview that put the meeting in historical perspective. Much to the surprise of the organizers, Slonimski pointed out that the timing coincided almost to the month with the 30th anniversary of Boris Ephrussi's announcement on the petite mutation (Ephrussi, B., Hottinguer, H., and Tavlitzki, *J. Ann. Inst. Pasteur* 76, 351, April 1949). In a series of seven articles, Ephrussi and his collaborators reported on many aspects of the petite mutation with only passing reference to DNA. Since this was some 14 years before the first demonstration of mitochondrial DNA, this was certainly not a startling omission. The ensuing years have shown that yeast mitochondria have occupied a signal position in elucidating the function of extrachromosomal DNA. As we shall see, the analysis of intervening genes is especially important in yeast mitochondria, as well as the sequencing of a variety of genes of known function.

This symposium witnessed the gathering of seemingly disparate groups of researchers involved in mitochondrial, chloroplast, plasmid, and viral DNA function and replication. As will be apparent, however, great similarities exist in these systems at both the molecular and phenomenological levels. In future years, these similarities may well lead to a more basic understanding of organelle evolution and biogenesis.

I want to thank my coorganizers Piet Borst, Igor Dawid, and Sherman Weissman for their enthusiasm and assistance in organizing this symposium. We also wish to thank the Life Sciences Division of ICN Pharmaceuticals, Inc., for their continued support of the ICN–UCLA Symposia series and the National Institutes of Health for contract #263-MD-912641 (jointly sponsored by the Fogarty International Center, National Cancer Institute and National Institute for Allergy and Infectious Diseases).

EXTRANUCLEAR GENETICS

G.H. Beale[1]

ABSTRACT A brief survey of the development of our knowledge of extranuclear genetics is presented. The material is grouped under three headings: (1) DNA-containing cell organelles; (2) endosymbionts, and (3) virus-like particles. The extremely uneven development of research on the different examples is pointed out. Comparisons between examples in the different groups are made regarding their DNA, the presence of histone-like proteins, and the relative control of extranuclear structures by nuclear and extranuclear genes. An attempt is made to establish homologies between members of different groups, and some evolutionary hypotheses are considered. It is suggested that use of the terms "prokaryote" and "eukaryote" may be inappropriate for extranuclear DNA and should be applied to whole cells or organisms. It is also pointed out that even the meaning of the word "extranuclear" has some obscurity. The need for research on a wider range of materials than have been studied hitherto is stressed.

INTRODUCTION

Extranuclear genetics is now such a large and diversified subject that it is well-nigh impossible to write a coherent account in the short space of a single contribution to a symposium. Nevertheless there are good reasons for making the attempt: it offers an opportunity to allow one's mind to roam over the whole field before concentrating on the minutia of particular examples. It shows that though a great deal has been learnt about certain parts of the subject, others have been seriously neglected. We know far more about mitochondria than about other extranuclear structures with genetic properties, except possibly some viruses, and amongst mitochondria, most of our information comes from one organism - Saccharomyces cerevisiae.

[1]Present address: Institute of Animal Genetics, West Mains Road, Edinburgh EH9 3JN, Scotland

ISBN 0-12-198780-9

To illustrate the variability of mitochondria amongst different organisms, the following facts should be noted. The DNA of yeast mitochondria is in the form of a circle of about 25 μm in circumference, while in other organisms the size may vary from 5 μm to 35 μm or more. In protozoa the mitochondrial DNA may be circular, linear or (in kinetoplasts) catenated (1). The mitochondrial ribosomes of different organisms also vary a great deal in size (from 55S in animals to 80S in plants or ciliates), and there are important differences in the genes (nuclear and mitochondrial) coding for the mitochondrial ribosomal proteins in yeast, Neurospora and Paramecium (2). Thus even within the one category of mitochondria a misleading impression is created if all conclusions are based on yeast, and still greater disparity is evident when one takes into consideration cytoplasmic structures other than mitochondria.

By contrast with this diversity of extranuclear genetic systems, the classical chromosomal mechanism controlling Mendelian heredity is remarkably uniform over the whole range of eukaryotic organisms, from protista to mammals, and there we were not led far astray by basing the whole theory on Drosophila, more or less. My aim therefore in presenting this paper is to draw attention to the diversity of extranuclear phenomena, and to the necessity of studying a wider range of materials.

To illustrate the development of our knowledge of extranuclear genetics, it is interesting to compare our present programme with the proceedings of a symposium held in Paris in 1948 (3). The earlier meeting was organised by André Lwoff and entitled "Unités biologiques douées de continuité génétique". At that meeting Ephrussi presented his first results on the "petite colonie" mutants of yeast, and tentatively ascribed their determination to a cytoplasmic,non-genic, factor. L'Héritier described his CO_2-sensitive strains of Drosophila, and showed them to be controlled by cytoplasmic elements which were called at that time "génoïdes". Rhoades discussed the plastids, which had long been known to show non-Mendelian properties - ever since the observations of Correns and Baur in 1909-, though in 1948 there was still a lot of argument about whether the non-Mendelian determinants were actually inside the chloroplasts, or somewhere else in the cytoplasm, or possibly comprised some ill-defined entity known as the "plasmon".

In the discussion of these and other non-Mendelian phenomena the word "plasmagene" was used by several speakers at the Paris meeting, as a kind of alternative to real chromosomal genes. Some participants speculated that there might be a whole range of sub-cellular particles, including

genes, plasmagenes, viruses and so on; and some people had doubts about the existence of extranuclear genes altogether. (I remember a postcard was written to Tracy Sonneborn, who did not attend the meeting, stating that "Les plasmagènes n'existent pas"). Delbrück put forward a hypothetical scheme based on a system of steady states involving enzymes and inhibiting metabolites, thus avoiding the need for any particulate determinant at all. This was before current ideas of protein synthesis based on translation of m-RNA had been put forward, and hence Delbrück's scheme could only be expressed in very general terms.

DNA was not referred to very much at the Paris meeting, except in connection with bacterial transforming principles, and this omission is not surprising since the theory of the double helix had not yet been conceived.

In the years between the Paris meeting and the present one, great progress has been made in certain areas, and no one any longer doubts the existence of extranuclear genetic determinants. Yeast and Chlamydomonas stand out as exceptionally favourable materials for this work and have been brilliantly exploited. However, some of the matters discussed in 1948 have not progressed very much, and some are not now thought to involve gene-line determinants. For example Lwoff discussed the notion of genetic continuity of kinetosomes - organelles situated just beneath the surface of ciliate cells. It is now thought that kinetosomes lack their own DNA and whatever hereditary properties they have (- and they certainly have something, as shown by the work of Beisson and Sonneborn (4)-) must be based on some other mechanism. Centrioles also, which resemble kinetosomes to some extent, are likewise probably devoid of gene-line components.

A crucial step in the development of our understanding of extranuclear genetics was the discovery, some 15 years after the Paris meeting, of DNA in mitochondria and plastids (5,6). This was the signal for many workers to claim immediately that organelle DNA had a genetic role, and conversely that presence of this DNA proved that structures containing it had genetic properties. It took some time however to substantiate these conjectures. I must confess that I found the discovery of organelle DNA rather depressing, since - seeking variety - some of us had previously hoped that some other system, not involving DNA, might emerge as a basis for extranuclear genetics. When this thought had to be relinquished, we faced the boring possibility that cytoplasmic genes would turn out to be merely nuclear genes in a new situation. After all DNA is DNA, always basically the same, chemically speaking.

This leads us to a question of current interest: is there a fundamental difference (organizational if not chemical) between nuclear and extranuclear DNA? I expect this question will be discussed later at this meeting. Whether the answer is "yes" or "no", however, it is now quite clear that extranuclear genetic particles show a number of idiosyncrasies which are not typical of nuclear genes. For example, with extranuclear genes there are usually numerous copies of a single structure in a single cell, whereas there are usually only one or two nuclear genes of a given kind in a cell; nuclear genes are arranged on a number of chromosomes, each individually distinct, whereas cytoplasmic genes are usually on a single structure, a circular piece of DNA; nuclear genes are subject to constraints imposed by the chromosomal mechanisms of mitosis and meiosis, which do not affect cytoplasmic genes; mutation of cytoplasmic genes shows differences from that of nuclear genes, and cytoplasmic genes may be transferred from cell to cell by infection and other mechanisms, unlike chromosomal genes. Many other differences could be listed.

Following this introduction, I plan to make an attempt to define what is meant by the word "extranuclear", and then classify the different types of extranuclear genetic phenomena, describing a few characteristics of each group. This will lead on to a discussion of problems of homology and evolution of extranuclear factors, and to consideration of the terms "prokaryotic" and "eukaryotic".

THE MAIN GROUPS OF EXTRANUCLEAR FACTORS

The problem of defining "extranuclear" factors turns out to be unexpectedly difficult. Obviously "extranuclear" means "outside the nucleus" and in eukaryotic cells the meaning is unambiguous (except possibly for some viruses and symbionts which are located within the nucleus, though separate from the chromosomes). In prokaryotes however there is no sharp boundary between the DNA-containing region (or nucleoid) and the rest of the cell: in *E. coli*, the prototype of all prokaryotes, there is a main DNA structure, often called "the chromosome", and in addition one or more smaller circles (plasmids, F factors, etc.) which may be considered as "extranuclear". However, some extranuclear components of eukaryotes (e.g. endosymbionts and organelles) may be considered homologous with the "nuclei" of prokaryotes, and if one accepts this, then a given type of structure might be "extranuclear" in one situation and "nuclear" in another, which is absurd. The problem is seen to be still more complicated when we consider the kappa particles of

Paramecium, which are located in the cytoplasm, and therefore "extranuclear" so far as Paramecium is concerned, but themselves contain virus-like particles. What are they? Extra-extranuclear? Here we have a kind of 3-tiered hierarchical system. If one wishes the definition to embrace both eukaryotes and prokaryotes, the word "extranuclear" can only refer to the position of one structure in relation to another structure denoted a "nucleus". I do not want to propose a more elaborate nomenclature inventing new terms, but merely wish to point out that we should face up to the reality of a complicated range of biological structures.

Extranuclear genetic factors may be put into three groups, as follows:

1. DNA-containing cell organelles,
2. endosymbionts, and
3. virus-like particles, including plasmids.

No doubt objections can be raised to lumping three lots of disparate materials in this crude way (especially in group (3)), but such a grouping is convenient for purposes of discussion. In group (1) there are only two examples: mitochondria and plastids, both of which are of course essential for life as it now exists on the earth. Group (2), the endosymbionts, comprises particles of a more restricted occurrence by comparison with organelles, though there are vast numbers and many different kinds of endosymbionts, and some are essential for the normal growth of their host organisms (e.g. the chlorophyll containing zoochlorellae of Paramecium bursaria, and the omikron particles of some freshwater Euplotes species (7)). As for group 3, the virus-like particles, they are so varied that one cannot say whether they are essential or not: many are of course pathological and therefore not essential at all to their host cells, but others e.g. some phages and the F factors of E. coli, play a role very closely integrated into that of the host bacterium. I would like to compare and contrast members of the three groups in regard to the following features: (1) their DNA; (2) the association of this DNA with basic proteins, and (3) the relative control by nuclear and extranuclear genes of extranuclear characters.

As for the DNA of members of the three groups, I mentioned above the variation in size and structural organisation of mitochondrial DNAs. Not much is known about the DNA of endosymbionts. Kappa particles have been reported to contain a main component consisting of long linear strands of DNA, and in addition small DNA circles of 13.75 μm contour length in the virus-like elements which are present, as already mentioned,within the kappa particles (8). Little work has been done on the DNA of other endosymbionts. Considering

viruses in general, there is of course a vast amount of information about virus and plasmid DNA, and it would be pointless to go into that here. It is however relevant to point out that some viruses and plasmids contain DNA circles roughly equivalent in size to those of mitochondria.

The determinants of the CO_2-sensitive strains of Drosophila, now denoted sigma viruses and known to be very similar to a known vertebrate virus (VSV), contain as a genetic element not DNA but RNA. Likewise the virus-like particles controlling another example of non-Mendelian genetics - the "killer" phenomenon in Saccharomyces and Ustilago, also contain RNA, this time double stranded (2). I suppose these two examples should be excluded from discussion at a symposium entitled "Extranuclear DNA", but we have to face the facts of life, and these particles are undoubtedly extranuclear genetic factors.

That is all I want to say about DNA, (and RNA as a genetic element), and I now turn to the question of the association of DNA with histones or histone-like proteins, such as occurs as a characteristic feature of eukaryotic chromosomes. Until recently it was generally accepted that the DNA of prokaryotes was "naked", though recently there have been reports of the occurrence of one or two histone-like proteins even in bacteria (9,10). However it has been suggested that the histone-like proteins of E. coli are present in too small an amount to complex E. coli DNA, as they do in eukaryotes forming nucleosomes (11). What is the situation in regard to mitochondrial DNA?

Formerly it was stated that mitochondrial DNA, like that of bacteria, was "naked", but this appearance was probably due to the drastic methods used in preparing the DNA. More delicate procedures have revealed the presence in mitochondria of Xenopus (12), Physarum (13) and Paramecium (14), of a beaded, chromatin-like structure. There is at present no certain proof that histones are present in these beads, but provisional study of the Paramecium material indicates that there may be four or five such basic proteins, which seem to be distinct from the histones in the nuclear chromatin of the same organism. We have no information about the genetic determination of these proteins: they could be nuclearly or mitochondrially coded, and it is hoped that this matter can be pursued in Paramecium which has certain technical advantages for studying the problem. Although this work is still at an early stage, present indications are that, in regard to possession of histone-like substances, and possibly nucleosomes, mitochondrial chromatin appears to have some eukaryotic features.

Nothing is known about histones in endosymbionts, so far

as I am aware. With regard to viruses, it should be noted that some animal viruses (adenovirus and SV-40) have the ability of associating with histones and forming nucleosome-like structures (15). This is discussed below.

The next matter which I would like to discuss is concerned with the relative importance of nuclear and extranuclear genes in the development and functioning of extranuclear structures. Members of each of the three groups referred to above are controlled by both nuclear and extranuclear genes, but the relative contributions of the two types of determinant vary a great deal in the different examples. We have extensive data about this matter for mitochondria, plastids and some viruses and plasmids, but very little for endosymbionts. Unfortunately, refined genetic analysis and gene mapping has not yet been achieved with any endosymbiont. However, there is crude evidence indicating that kappa particles and other ciliate endosymbionts are largely governed by their own genes, and relatively little by genes in the ciliate nuclei (2). An indication that this is so is given by the finding that some of these endosymbionts (though so far not kappa) have been cultured in a synthetic medium.

By contrast, cell organelles are largely governed by nuclear genes, though organelle genes are certainly essential too. Perhaps 90% of gene products in mitochondria are coded in the nucleus, and only 10% in the mitochondrial DNA. No one has cultured mitochondria in vitro, and I don't imagine this will be done for a long time, if ever.

What is remarkable about the genetic control of cell organelles is the extraordinarily tight integration of the products of nuclear and organelle gene activity. The replication and transcription of mitochondrial DNA is largely controlled by proteins coded in nuclear DNA; the translational mechanims comprising r-RNA, ribosomal proteins and t-RNAs, etc., involves an extremely intimate association of nuclear and extranuclear gene products, and the same applies to some proteins in the inner membranes, such as ATP-ase, cytochrome oxidase, etc. A similar situation exists in chloroplasts, where the major protein (fraction I or ribulose bisphosphate carboxylase)is a mixture of nuclear and plastid gene products. Thus there is an intricate mixing of nuclear and extranuclear gene products in many parts of the organelles.

As for the third group - the virus-like particles - there is too great a variety amongst them for us to be able to make a general statement concerning the relative importance of host and virus genes. However in some cases (e.g. bacterial plasmids) there is overriding control by the bacterial genome (15) and the plasmid genome does very little except determine the structure of replicas of itself. In the most extreme

situation where there is integration of the plasmid in the bacterial chromosome, the plasmid practically loses its identity altogether.

Thus, in a very general way one can arrange the three groups of determinants in the following order: endosymbionts showing most control by their own genes, and relatively little by the nuclear genes of the host cell; organelles showing a preponderance of control by nuclear genes, but an intimate interaction between nuclear and organelle gene products; virus-like particles showing - in some cases - the most extreme situation of almost total control by the host genome, though of course there are many exceptions to these statements.

HOMOLOGIES AND EVOLUTION OF EXTRANUCLEAR STRUCTURES

The three groups - organelles, endosymbionts and viruses - are so dissimilar that it may seem pointless to try and establish homologies between them, even in an evolutionary sense. While it is true that all - or nearly all - contain DNA which can be called extranuclear, this might be their only common feature. However, there are a number of considerations - mainly of a speculative nature - indicating relationships between members of the different groups. I will briefly mention a few, at random.

1. If the symbiotic theory (16) for the evolution of organelles is true (and of course it may not be), mitochondrial and bacterial endosymbionts have a common origin, as have chloroplasts and blue-green algae.
2. As Raff and Mahler (17) have hypothesized, mitochondrial DNA may have originated from a plasmid.
3. Mitochondria have a number of virus-like properties, e.g. the size of their DNA circles, and their mechanism of recombination, (where it occurs).
4. Viruses occur in some endosymbionts.

Other inter-connections could certainly be traced. Nevertheless to attempt to establish an evolutionary order amongst this welter of extranuclear structures is a daunting task. It is perhaps simplest to start with the endosymbionts, many of which show resemblances to known free-living microorganisms. For example, kappa shows a number of bacterial characteristics, and has been awarded a bacterial, binomial designation (Caedobacter taeniospiralis)(18). Other endosymbionts resemble rickettsiae, spiroplasma, green algae and other microbial groups (2). Some are naturally or artificially infectious and there seems no conceptual difficulty in accepting them as descendants of various

microorganisms.

No such easy solution is offered to us for the evolution of cell organelles, which in my view are very distinct from any known free-living organism. Two distinct hypotheses have been proposed (17,18), the symbiotic and non-symbiotic, as well as a number of variants of each. In connection with these hypotheses there has been much discussion of the supposed prokaryotic or eukaryotic nature of cell organelles. Formerly the prokaryotic features were stressed, e.g. the 'naked' DNA,which I have discussed above, and which was thought to be characteristic of organelles and bacteria; and the sensitivity of organelle ribosomes to antibiotics inhibiting ribosomes of bacteria but not of higher organisms. Recently opinion is veering in the opposite, i.e. eukaryotic, direction (19), as facts have accumulated showing presence of repetitive DNA sequences and of "split" genes in organelle DNA, poly-A on organelle m-RNA, and finally the possibility of nucleosome-like structures on organelle DNA as mentioned above.

It seems to me however that discussion as to whether organelle DNA is prokaryotic or eukaryotic is rather futile, since any structure in a eukaryotic cell is liable to show some eukaryotic features. Even some viruses (adenovirus and SV 40), when in animal cells, are capable of forming associations with four histones and form nucleosome-like beads; and the same viruses show leader or spliced segments of m-RNA (15). So far as I know, no one has described these viruses as "eukaryotic". It seems likely that cell organelles, like viruses inhabiting eukaryotic cells, are able to exploit the production by the host cell (ultimately from its nuclear genes) of certain enzymes which are required for such processes as the excision and ligation of the m-RNA produced by split genes, or the attachment of poly-A to messenger, or other "eukaryotic" functions. If we are right in believing that mitochondria contain histones, the latter could be coded by the nuclear genome and transported into the organelles. Thus all eukaryotic features of organelles or viruses could be due, ultimately, to the genetic capabilities of the host cell.

In my view, the terms "prokaryotic" and "eukaryotic" should not be applied to DNA, or even to individual constituents of cells, but to larger structures, whole cells, with their heterogeneous contents of nuclei, cytoplasm, organelles etc. This line of argument does not help us establish the homologies and evolutionary history of organelles, but perhaps might have the advantage of compelling us to seek alternative hypotheses.