

# **Nuclear structures**

**Isolation and characterization**

*Edited by*

**A. J. MacGillivray**

*and*

**G. D. Birnie**

# **Nuclear structures**

Isolation and characterization

*Edited by*

**A. J. MacGillivray**

Reader in Biochemistry

School of Biological Sciences, University of Sussex

*and*

**G. D. Birnie**

Senior Scientist

Beatson Institute for Cancer Research, Glasgow

**Butterworths**

London · Boston · Durban · Singapore · Sydney · Toronto · Wellington

All rights reserved. No part of this publication may be reproduced or transmitted in any form or by any means, including photocopying and recording, without the written permission of the copyright holder, application for which should be addressed to the Publishers. Such written permission must also be obtained before any part of this publication is stored in a retrieval system of any nature.

This book is sold subject to the Standard Conditions of Sale of Net Books and may not be re-sold in the UK below the net price given by the Publishers in their current price list.

First published, 1986

© Butterworth & Co. (Publishers) Ltd, 1986

**British Library Cataloguing in Publication Data**

Nuclear structures: isolation and characterization

1. Cell nuclei

I. MacGillivray, A. J.      II. Birnie, G. D.

574.87'32      QH595

ISBN 0-407-00323-1

**Library of Congress Cataloging in Publication Data**

Main entry under title:

Nuclear structures.

Includes bibliographies and index.

1. Cell nuclei—Separation.    2. Cell organelles.

3. Cell fractionation.    4. Cytochemistry.

I. MacGillivray, A. J.    II. Birnie, G. D.

QH595.N83    1986    574.87'32'028    85-26982

ISBN 0-407-00323-1

Cover illustration adapted from figure 2, drawn by Linda A. Buchholtz, in 'Dynamic Properties of the Nuclear Matrix' by Ronald Berezney in *The Cell Nucleus*, Vol. VII, *Chromatin Part D*, edited by Harris Busch, © 1979 by Academic Press, Inc. Reproduced by permission of the publisher.

# Preface

During the decade since the publication of *Subnuclear Components*, as techniques for the isolation of molecular and macromolecular constituents of cell nuclei improved, the emphasis in research has shifted from individual molecular components to identifiable structures such as the nuclear envelope, ribonucleoprotein particles, etc. The present book is designed to reflect not only current interests in the structures now recognized to exist in the nuclei of cells, but also changes and advances in preparative techniques for nuclear components and certain macromolecules. We are of the opinion that, in view of the movement of interests and research, the publication of this book will be timely; moreover, to the best of our knowledge, no other text covers these aspects.

The overall objective of this book is to provide reasoned assessments of current techniques used to isolate and characterize nuclear structures, together with 'best buys' in recommended procedures. To achieve these ends we have persuaded each of our authors to use their experience and judgement in their field to review available procedures, to recommend particular methods, and to give reasons for their choices. It is regrettable that precise technical details (for example, conditions of centrifugation) are not always given in today's scientific papers and we have specifically asked for such essentials to be included. As a result, technical details are omitted from the text of this book only in exceptional circumstances, for example when a contributor, in making comparisons of experimental approaches, has had to rely solely on the information provided in an original article. In addition, each contributor has provided an indication of the evaluation of their end-product. Consequently, a degree of difference in balance between background material and detailed technical information exists throughout the book; this is merely a reflection of how different areas have progressed to date.

We appreciate that not all known nuclear structures are dealt with in the chapters to follow. Some are omitted simply because no particular advances have occurred in their preparation during the past ten years. The methodology to isolate others is not as yet at a stage that is suitable for discussion, for example the components of the mitotic apparatus other than the chromosomes themselves. The isolation of nuclei from cells is, of course, of prime importance, but again no major advances in these methodologies have appeared in recent years. Each author has, however, been asked to quote their preferred procedure for preparing nuclei. Although the chapters in part act as current reviews of specific areas of the biochemistry and molecular biology of the cell nucleus, the thrust of the book is a practical one. In

this, it is our belief that an approach based on critical comparisons between methodologies and detailed explanations of procedures recommended avoids the production of what would otherwise be little more than a compendium of recipes, lacking guidance as to what menu is appropriate to a particular situation.

We must express our gratitude to our authors for their cooperation and understanding, particularly during lengthy discussions over the final format of their contributions. We are also indebted to the staff of Butterworths Scientific Ltd for help, advice and encouragement throughout the preparation of the book.

A. J. MacGillivray  
G. D. Birnie

# Contributors

**Caroline V. P. Addey,**

Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ

**Paul S. Agutter,**

Department of Biological Sciences, Napier College, Colington Road, Edinburgh EH10 5DT

**J. Allan,**

Department of Biophysics, King's College London, University of London, 26–29 Drury Lane, London WC2B 5RL

**Trevor J. C. Beebee,**

Biochemistry Laboratory, School of Biological Sciences, University of Sussex, Brighton BN1 9QG

**George D. Birnie,**

Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Bearsden, Glasgow G61 1BD

**Ailsa M. Campbell,**

Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ

**Shona A. Comerford,**

Department of Biological Sciences, Napier College, Colington Road, Edinburgh EH10 5DT

**G. H. Goodwin,**

Chester Beatty Laboratories, Institute of Cancer Research, Fulham Road, London SW3 6JB

**Sheila V. Graham,**

Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Bearsden, Glasgow G61 1BD

**Z. Islam,**

Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ

**Dean A. Jackson,**

Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE

**J. T. Knowler,**

Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ

**Robin Leake,**

Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ

**Alexander J. MacGillivray,**

Biochemistry Laboratory, School of Biological Sciences, University of Sussex, Brighton BN1 9QG

**C. W. McGregor,**

Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ

**Alexander G. McLennan,**

Department of Biochemistry, University of Liverpool, PO Box 147, Liverpool L69 3BX

**R. H. Nicolas,**

Chester Beatty Laboratories, Institute of Cancer Research, Fulham Road, London SW3 6JB

**Bryan D. Young,**

Medical Oncology Unit, Imperial Cancer Research Fund Laboratories, PO Box 127, Lincoln's Inn Fields, London WC2A 3PX



# Contents

Preface v

Contributors vii

## 1 Nuclear matrices 1

*Shona A. Comerford, Paul S. Agutter and Alexander G. McLennan*

Isolation of nuclear matrices 2

Conclusions 9

Notes on the methods 9

Acknowledgements 12

References 12

## 2 Nucleoids 14

*Dean A. Jackson*

Nucleoid preparation and morphology 14

Preparation of nucleoids from HeLa cells 14

HeLa nucleoid morphology 15

Preparation of nucleoids from other cell types 18

Procedures for stabilizing nucleoids 23

Characterization of nucleoids 23

Proteins 23

Enzyme activities 24

DNA 26

RNA 28

Experimental uses of nucleoids 29

Criticism of nucleoid preparation 30

Conclusions 31

Acknowledgement 32

References 32

## 3 Nuclear envelopes 34

*Paul S. Agutter*

Background 34

Objectives of nuclear envelope isolation 34

General constraints	35
Development of isolation procedures to meet different research objectives	36
Procedures	36
High ionic strength procedures	36
Low ionic strength procedures	39
General considerations	41
Notes on the methods	43
References	45
<b>4 Polynucleosomes and monomer nucleosomes</b>	<b>47</b>
<i>J. Allan, R. H. Nicolas and G. H. Goodwin</i>	
Isolation of polynucleosomes	48
General points on the preparation of polynucleosomes	49
The preparation of chicken erythrocyte polynucleosomes	56
Fractionation of monomer nucleosomes	59
Isolation of salt-soluble nucleosomes	60
Electrophoresis of nucleosomes	61
Analysis of electrophoretically separated nucleosomes	63
Evaluation of methods used to isolate transcriptionally active nucleosomes	68
Acknowledgements	71
References	71
<b>5 Metaphase chromosomes</b>	<b>74</b>
<i>Bryan D. Young</i>	
Bulk preparation of metaphase chromosomes	75
Details of procedures	75
Discussion of methods	77
Flow cytometry of metaphase chromosomes	79
Choice of DNA-specific stain	79
Technical innovations	80
Applications of chromosome fractionation	81
Flow karyotype analysis	81
Gene mapping	82
Chromosomal DNA library construction	83
Acknowledgements	84
References	84
<b>6 DNA tight-binding proteins</b>	<b>86</b>
<i>Ailsa M. Campbell and Caroline V. P. Addey</i>	
The definition of a DNA tight-binding protein	86
Definition based on analogy with bacterial systems	86
Operational definition based on isolation procedures	86
Operational definition based on specific DNA binding	87
The preparation of DNA tight-binding proteins	87
Isolation of nuclei	87
Isolation of chromatin	89
Extraction of DNA tight-binding proteins	89

Immunological characterization of tight-binding proteins	92
Methods of immunoassay	92
DNA binding characteristics	95
Detection of sequence specific DNA binding proteins	96
Conclusions	98
Acknowledgements	98
References	98
<b>7 Nucleoli and preribosomal ribonucleoprotein particles</b>	<b>100</b>
<i>Trevor J. C. Beebee</i>	
Methods for the isolation of nucleoli	100
Isolation using ultrasonication	100
Isolation using homogenization	102
Other methods	104
Assessment of nucleolar preparations	106
Yield and purity	106
Internal integrity	106
Conclusions	111
Isolation of preribosomal ribonucleoprotein particles	112
Methods	112
Assessment of preparations	113
Overview	116
Acknowledgements	116
References	116
<b>8 Ribonucleoprotein particles containing heterogeneous nuclear RNA</b>	<b>118</b>
<i>J. T. Knowler, C. W. McGregor and Z. Islam</i>	
Isolation of hnRNP	118
Isolation of hnRNP by extraction from nuclei	118
Isolation of hnRNP by nuclear lysis	120
Isolation of monomeric versus polymeric hnRNP	122
Characterization of hnRNP	122
The purity and authenticity of isolated hnRNP	122
Further purification of hnRNP: the problem of desirability	124
The fractionation and characterization of hnRNP proteins	124
Acknowledgement	128
References	128
<b>9 Nuclear RNP particles containing small RNAs</b>	<b>130</b>
<i>Alexander J. MacGillivray</i>	
Background, objectives and limitations to the preparation of snRNP particles	131
Procedures using immunoaffinity chromatography	135
Preparation of snRNP particles containing U1 and U2,4-6 snRNAs	135
Preparation of La-RNP	139
Immunoaffinity chromatography using autoantibodies – conclusions	142
Use of antibodies to 2,2,7-trimethylguanosine to isolate snRNP particles	143

Procedures using non-immunological techniques	148
Isolation of total snRNP particles and their partial fractionation	148
Preparation of U1, U1-2 and U4-6 snRNP particles	150
Preparation of U1 and U2 snRNP particles	154
General conclusions	157
Acknowledgements	160
References	160

## **10 Hormone receptors 163**

*Robin Leake*

Peptide hormones	163
Thyroid hormones	164
Steroid hormones	164
The classic model	165
The equilibrium model	166
A new model of steroid receptor action	169
Properties of receptors for individual steroids	171
Assay methods	175
Acknowledgements	178
References	179

## **11 DNA and RNA 182**

*G. D. Birnie and S. V. Graham*

Isolation of nuclei	183
Need for isolated nuclei	183
Rationales of protocols	184
Fractionation of cells	185
Preparation of purified nuclei	187
Isolation of nucleic acids	189
DNA	189
RNA	191
Polyadenylated RNA	194
Assessment of preparations	196
Nuclei	196
DNA	197
RNA	198
Acknowledgements	200
References	200

## Nuclear matrices

Shona A. Comerford, Paul S. Agutter and Alexander G. McLennan

More than 40 years ago, it was shown that a subfraction of nuclear proteins resisted extraction with buffers of high ionic strength (Mayer and Gulick, 1942). During the 1950s and 1960s, evidence accumulated that this salt-resistant fraction represented a definite intranuclear protein or ribonucleoprotein fibrillar network (for reviews see Berezney and Coffey, 1976; Agutter and Richardson, 1980). The first detailed isolation procedure for this structure, the nuclear matrix, was published by Berezney and Coffey (1974) and since then both the validity of this procedure and the physiological reality of the matrix have been controversial issues. The susceptibility of nucleoplasmic constituents to artefactual precipitation under a variety of conditions has been emphasized (see e.g. Skaer and Whytock, 1977; Kaufmann, Coffey and Shaper, 1981; Laemmli, Lewis and Lebkowski, 1981), and it is clear that while Berezney and Coffey (1974) isolated an intranuclear fibrillar structure, other workers using apparently similar techniques (nuclease digestion, extraction with high salt concentrations and treatment with non-ionic detergents) isolated only the peripheral pore-complex-lamina (Aaronson and Blobel, 1975; see also Chapter 3 in this volume).

We take the view that more recent electron microscopic evidence (e.g. Brasch, 1982; Guatelli *et al.*, 1982; Capco, Wan and Penman, 1982; Diaz de la Espina *et al.*, 1982), taken together with earlier studies, establish the physiological reality of the matrix beyond reasonable doubt, and that the immediate task is to obtain an isolated preparation that corresponds ultrastructurally to the *in situ* network described in these publications. Our view is not universally held, however, and other workers would consider that the controversy about the existence of the matrix is not yet settled.

The persistence of this controversy seems to us to have resulted from the poor characterization of the matrix to date. In contrast to the well-established components of the cytoskeleton, 'nuclear matrices' are ultrastructurally and biochemically ill-defined. Ultrastructurally, almost all preparations comprise irregular, heterogeneous, broad, rather amorphous fibres rather than regular, homogeneous, fine, well-formed ones. Biochemically, their main specific polypeptide components (other than lamins – see Chapter 3) have not been identified; antibodies that react only with the postulated intranuclear fibrils *in situ* have not been obtained, though recent findings by Chaly *et al.* (1983) may resolve this problem, and *in vitro* reconstitution has not been accomplished. We believe

that until these deficiencies are remedied, no real progress can be made with the potentially richly rewarding studies of the role of the matrix in a number of major nuclear functions such as DNA replication (Berezney and Coffey, 1975), RNA processing (Wunderlich, Berezney and Kleinig, 1976), steroid hormone binding (Agutter and Birchall, 1979; Barrack and Coffey, 1980), viral replication (Hodge *et al.*, 1977; Chin and Maizel, 1977) and carcinogenesis (Zbarsky, Dmitrieva and Yermolayeva, 1962; Hemminki and Vainio, 1979).

The confusion of the last decade has been exacerbated by attempts to pursue such studies despite the poor state of basic characterization of the structure, by the concomitant proliferation of 'isolation' procedures and by inconsistencies in nomenclature. (The term 'matrix' has been used specifically to describe an intranucleoplasmic fibrillar system, and more generally to refer to whole chromatin-depleted nuclear residues comprising pore-complex-laminae and nucleolar residues together with such fibrils. We shall use the term in the latter, general sense. Moreover, such phrases as 'nuclear skeleton', 'nuclear ghost' and 'nuclear cage' have been used to describe analogous preparations. We shall avoid these terms, but it should be emphasized that studies of the 'nuclear cage' have thrown valuable light on the mechanism of DNA replication. (See Chapter 2.)

In this chapter, we discuss types of isolation procedures for the matrix and also a series of studies which made an important contribution to clarifying the issues involved in matrix isolation. Other published procedures for matrix isolation are variants of those treated here. Discussion of these five major procedures and their derivatives seems to us to provide a reasonable overview of the evolution of matrix isolation techniques, and in doing so it throws some light on the development of the controversy surrounding the existence of the structure *in vivo*. We must emphasize that although a satisfactory isolation procedure might now be available, such detailed biochemical characterization as is necessary for further progress is still far from completed.

## Isolation of nuclear matrices

### The method of Berezney and Coffey (1974)

This method was originally designed for the preparation of rat liver nuclear matrices. Later it was used to isolate matrices from other mammalian tissues, including endometrium (Barrack *et al.*, 1977) and lung (Agutter and Birchall, 1979). Its importance lies partly in this apparent versatility, partly in its role in initiating the controversy about the matrix and partly in the fact that procedures that were developed subsequently were derived from it, or at least influenced by it.

**Step 1** Rat liver (50 g) is minced and homogenized in 200 ml of STM (50 mM tris-HCl, 5 mM MgCl<sub>2</sub>, 250 mM sucrose, pH 7.4 at 4°C) and filtered through cheesecloth. The homogenate is centrifuged at  $780 \times g_{\max}$  (1600 rev/min in the 12  $\times$  100 ml rotor of a MSE Coolspin centrifuge) for 10 min at 4°C and the pellet is resuspended in STM adjusted to 2.2 M sucrose. The nuclei obtained after centrifugation at  $40\,000 \times g_{\max}$  (16 500 rev/min in the 10  $\times$  100 ml rotor of a MSE 50 centrifuge) for 90 min at 4°C are washed (*see* Note 1, p.9) twice in STM. All subsequent operations except nuclease digestion are performed at 0°C in 10 mM tris-HCl, pH 7.4 (adjusted at 0°C).

**Step 2** The nuclei obtained by the above procedure are lysed by resuspension in 20 vol. of 10 mM tris-HCl, pH 7.4 buffer containing 0.2 mM MgCl<sub>2</sub>, incubation for 10 min and centrifugation at  $780 \times g_{\max}$  (as above) for 20 min. This step is repeated once.

**Step 3** The pellet is resuspended in 10 mM tris-HCl, pH 7.4 buffer containing 0.2 mM MgCl<sub>2</sub>, 2.0 M NaCl. After incubation for 10 min the suspension is centrifuged at  $780 \times g_{\max}$  for 40 min. This step is repeated twice.

**Step 4** The chromatin-depleted pellet is resuspended in 10 vol. of 1% (v/v) Triton X-100, 5 mM MgCl<sub>2</sub> in 10 mM tris-HCl, pH 7.4 buffer and incubated for 10 min. The membrane-depleted nuclei are recovered by centrifugation at  $780 \times g_{\max}$  for 20 min. This step is not repeated.

**Step 5** Residual nucleic acids are removed by incubation with 200  $\mu$ g DNAase I, 200  $\mu$ g RNAase A in 1 ml of 5 mM MgCl<sub>2</sub> at 22°C for 1 h. The matrices are then sedimented by centrifugation at  $780 \times g_{\max}$  and are washed twice in 5 mM MgCl<sub>2</sub>. (For discussion of the most important features of this method, see Notes 2 and 3, p.10.)

### The method of Wunderlich and Herlan (1977)

This method was designed to isolate matrices from the macronuclei of *Tetrahymena pyriformis*. The Ca<sup>2+</sup>/Mg<sup>2+</sup> ratios used are apparently appropriate to the internal environment of this organism; as the total concentrations of these cations increase and decrease, the matrix respectively contracts and expands (Herlan, Quevedo and Wunderlich, 1978).

**Step 1** The isolated nuclei from  $10^9$ – $10^{10}$  cells are incubated for 10 min, 4°C in 4–5 vol. of 20 mM tris-HCl, 2 mM MgCl<sub>2</sub>, 3 mM CaCl<sub>2</sub>, 0.1 mM ATP, 0.3% (v/v) Triton X-100, 1% (w/v) polyvinylpyrrolidone K90, pH 7.4, and centrifuged at  $900 \times g_{\max}$  (1700 rev/min in the 12  $\times$  100 ml rotor of a MSE Coolspin centrifuge) for 10 min at 4°C.

**Step 2** After washing (see Note 1, p.9) in LCMT (20 mM tris-HCl, 2 mM MgCl<sub>2</sub>, 3 mM CaCl<sub>2</sub>, 0.1 mM ATP, pH 7.4) they are incubated with 100  $\mu$ g DNAase I, 100  $\mu$ g RNAase A in 5 ml of HCMT (i.e. LCMT adjusted to 50 mM MgCl<sub>2</sub> and 75 mM CaCl<sub>2</sub>) for 75 min at 20°C.

**Step 3** The nuclease action is terminated by the addition of 9 vol. (45 ml) of LCMT followed by centrifugation at  $1300 \times g_{\max}$  (2000 rev/min in the 12  $\times$  100 ml rotor of an MSE Coolspin centrifuge) for 20 min at 4°C.

**Step 4** The nuclease digestion is repeated under the same conditions for 30 min in HCMT and is terminated as before. Finally, the matrices are washed (see Note 1, p.9) twice in HCMT. The yield of matrix material obtained by this method is discussed in Note 4, p.10, and implications for the universality of the matrix are discussed in Note 5, p.10).

### The method of Long, Huang and Pogo (1979)

At the time of this publication, the interests of Pogo and his co-workers centred on the organization of intranuclear ribonucleoprotein in cultured cells. The method is designed specifically for isolating RNA-rich matrices from Friend erythroleukaemic cells, but was derived from earlier studies by the same group (*see e.g.* Faiferman and Pogo, 1975; Miller, Huang and Pogo, 1978). In Note 6 (p.10) and in the following text, the implications of the RNA content of the isolated structure are discussed. In Note 7 (p.11) the possibility of applying the same or a very similar procedure to other cell types is discussed; specifically, the work of van Venrooij and his colleagues (van Eekelen and van Venrooij, 1981) on HeLa cell matrices is examined.

*Step 1* The cells are washed twice with 10 mM PIPES, 100 mM KCl, 1.5 mM MgCl<sub>2</sub>, 146 mM sucrose, pH 7.0 (adjusted at 23°C). In this context, 'washed' is to be understood not as in Note 1, but as replacement of the culture medium by an equal volume of the buffer.

*Step 2* Lysis of the cells is achieved using the same medium supplemented with 0.5 mM phenylmethylsulphonyl fluoride (PMSF) (*see* Note 6, p.10), 0.25% (v/v) Triton X-100 and 0.25% (w/v) saponin. To facilitate lysis, repeated passage (10–20 times) through a plastic pipette is recommended by the authors (Note 8, p.11).

*Step 3* Differentiated nuclei are sedimented at  $250 \times g_{\max}$  (900 rev/min in the 12  $\times$  100 ml rotor of a MSE Coolspin centrifuge), and undifferentiated nuclei at  $500 \times g_{\max}$  (1250 rev/min in the same rotor), for 10 min at 4°C.

*Step 4* After washing (Note 1) twice in 10 mM tris-HCl, 100 mM KCl, 5 mM MgCl<sub>2</sub>, 0.5 mM CaCl<sub>2</sub>, 146 mM sucrose, 0.5 mM PMSF, pH 7.7 (adjusted at 23°C), the nuclei are resuspended to a concentration of  $1.5 \times 10^8$ /ml in the same buffer supplemented with 500  $\mu$ g/ml of DNAase I (*see* Note 9, p.11) and are incubated at 10°C for 60 min (undifferentiated) or 120 min (differentiated nuclei).

*Step 5* After this incubation, the suspension is layered over 20–25 ml of 1 M sucrose in 10 mM tris-HCl, 100 mM KCl, 1.5 mM MgCl<sub>2</sub>, pH 7.7 (adjusted at 23°C) and centrifuged at  $6000 \times g_{\max}$  (6000 rev/min in the 6  $\times$  14 ml rotor of a MSE Europa 50 centrifuge) for 10 min at 4°C. The matrices pellet.

*Step 5* of this procedure can be modified in two ways. It can be replaced by simple sedimentation as described for the nuclei, in which case the pellet is rich in chromatin fragments (this might be useful if interactions between the matrix and DNA or histones is being studied). Alternatively, the KCl concentration can be increased to 0.5–1.5 M, in which case the extent to which DNA and histones are extracted is increased correspondingly.

In either case, however, the recovery of nuclear RNA in the matrices is close to 100%. This raises an interesting and important issue because ribonuclease treatment has been found to disrupt the intranuclear matrix fibrils. Thus, while the findings from early applications of the methods described on p.2 *et seq.* of this chapter seemed to point to a more or less wholly proteinaceous fibrillar matrix, the results obtained by Long, Huang and Pogo (1979) recalled earlier indications that the intranuclear ground substance is a network comprising both protein and RNA



(Smetana, Steele and Busch, 1963; Narayan *et al.*, 1967; Faiferman and Pogo, 1975). This emphasized an additional aspect of the controversy surrounding the reality and nature of the matrix: how dependent is the integrity of the structure on RNA?

### The studies of Kaufmann, Coffey and Shaper (1981)

Although the work of Shaper and his colleagues was not intended to lead to a new method for isolating matrices, it elucidated the mechanisms underlying the procedures previously used and has played a crucial part in initiating subsequent methodological developments. Therefore, we summarize its principal conclusions, here and urge the reader who is interested in matrix isolation to study this key paper in the field with particular care.

Kaufmann, Coffey and Shaper began with the question: why did Berezney and Coffey (1974) and Aaronson and Blobel (1975), using apparently identical techniques and the same starting material, obtain such markedly different results? (See p.2 *et seq.* and Note 3, p.10), for details.) When they attempted to shorten the operation time of the Berezney-Coffey procedure, treating the nuclei initially with DNAase and RNAase to avoid overnight incubation (cf. Berezney and Coffey, 1977) but using the same buffers as Berezney and Coffey, supplemented with PMSF (see p.2), Kaufmann, Coffey and Shaper obtained a far less extensive intranuclear network and no identifiable nucleolar residues. Further investigation revealed that structures of very different compositions and morphologies could be isolated from the same batch of nuclei, if the order and speed of the extraction steps were varied slightly.

The main conclusions were first, when RNAase treatment is used *after* high-ionic-strength extraction, intranuclear matrix fibrils and nucleolar residues can be retained. When it is used *before* such extraction, intranuclear structure is largely absent and only empty spheres of nuclear envelope persist. (See Note 6, p.10).

Secondly, the stability of the intranuclear fibrils is dependent on the oxidative crosslinking of proteins with endogenous sulphhydryl groups. If such crosslinking is made extensive at the pure-nuclei stage, for example by overnight incubation, by treatment with sodium tetrathionate or by cationic detergents, then even RNAase treatment before high-salt extraction does not disrupt the matrix. If matrices are obtained by, for instance, the method of Berezney and Coffey (1977), then subsequent treatment of them with 1% (v/v) 2-mercaptoethanol in high-salt buffer destroys the internal structure and leaves only hollow spheres of nuclear envelope (see Notes 2 and 3, p.10).

Thirdly, reproducibility of the findings, including recovery of nuclear protein and RNA, depends on the inclusion of PMSF in all the buffers used, including those used for isolating nuclei. Other serine protease inhibitors are less satisfactory. (In our hands, phenylmethylsulphonylchloride (PMSC) is as effective as PMSF; cf. Agutter, 1983). (See Notes 2 and 7, pp.10,11.)

Fourthly, extraction with solutions containing Triton X-100 leads to aggregation of the material, and therefore more satisfactory matrix preparations are obtained if the nuclear membranes are not removed by detergent treatment during isolation.

On the basis of these findings, it seems appropriate to use PMSF or PMSC throughout the isolation procedure and to avoid detergent extraction. Moreover, the stability of the matrix structure seems to depend both on the integrity of the