Methods in Cell Biology

VOLUME XVI

Chromatin and Chromosomal Protein Research. I

Edited by

GARY STEIN and JANET STEIN

LEWIS J. KLEINSMITH

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PREFACE

During the past several years considerable attention has been focused on examining the regulation of gene expression in eukaryotic cells with emphasis on the involvement of chromatin and chromosomal proteins. The rapid progress that has been made in this area can be largely attributed to development and implementation of new, high-resolution techniques and technologies. Our increased ability to probe the eukaryotic genome has far-reaching implications, and it is reasonable to anticipate that future progress in this field will be even more dramatic.

We are attempting to present, in three volumes of *Methods in Cell Biology*, a collection of biochemical, biophysical, and histochemical procedures that constitute the principal tools for studying eukaryotic gene expression. Contained in this volume (Volume 16) are methods for isolation of nuclei, preparation and fractionation of chromatin, fractionation and characterization of histones and nonhistone chromosomal proteins, and approaches for examining the nuclear-cytoplasmic exchange of macromolecules. Volume 17 deals with further methods for fractionation and characterization of chromosomal proteins, including immunological, DNA affinity, and sequencing techniques. Also contained in Volume 17 are methods for isolation and fractionation of chromatin, nucleoli, and chromosomes. The third volume (Volume 18) focuses on approaches for examination of physical properties of chromatin, enzymic components of nuclear proteins, chromatin transcription, and chromatin reconstitution. Volume 18 also contains a section on methods for studying histone gene expression.

In compiling these three volumes we have attempted to be as inclusive as possible. However, the field is in a state of rapid growth, prohibiting us from being complete in our coverage.

The format generally followed includes a brief survey of the area, a presentation of specific techniques with emphasis on rationales for various steps, and a consideration of potential pitfalls. The articles also contain discussions of applications for the procedures. We hope that the collection of techniques presented in these volumes will be helpful to workers in the area of chromatin and chromosomal protein research, as well as to those who are just entering the field.

We want to express our sincere appreciation to the numerous investigators who have contributed to these volumes. Additionally, we are indebted to Bonnie Cooper, Linda Green, Leslie Banks-Ginn, and the staff at Academic Press for their editorial assistance.

GARY S. STEIN
JANET L. STEIN
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Erratum

METHODS IN CELL BIOLOGY, Vol. 16

Page 450 lines 15–17 should read:
matin is found in the interphase which is not collected; however, if chromatin is prepared by other methods this could vary and should be carefully controlled. The amount of chromatin in the upper and lower phases is deter-

Part A. Isolation of Nuclei and Preparation of Chromatin. I

Chapter 1

Methods for Isolation of Nuclei and Nucleoli

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I. Isolation of Nuclei

Procedures for the isolation of nuclei have been important in studies on histones and other nuclear proteins (*1, 2*), nuclear enzymes (*2–6*), and nuclear RNA and DNA (*7, 8*). Since nuclei are a starting material for isolation of nucleoli, procedures for isolation of nuclei must provide products that are satisfactory with respect to yield, morphology, and chemical composition, if isolation of nucleoli is to be satisfactory.

The objectives of procedures for isolation of nuclei are to secure a product in which (a) the nuclei are morphologically (Fig. 1) identical to those of the whole cell, (b) the contents of the nuclei as they exist in the cell are all present in the isolated product, and (c) the isolated nuclei do not contain cytoplasmic constituents. At the present time, the procedures commonly used for isolation of nuclei are (1) modification of the procedure of Chauveau *et al.* (*9*) in which the tissue is homogenized in sucrose solutions containing Ca^{2+} followed by centrifugation of the homogenate [another procedure used is the nonaqueous technique in which the tissue is subjected to rapid freezing and lyophilization followed by a milling procedure in nonaqueous solvents (*10,11*)]; (2) treatment of the tissue with citric acid following the technique of Dounce and his associates (*12–15*); and (3) techniques employing "hypotonic shock" followed by treatment with detergents and

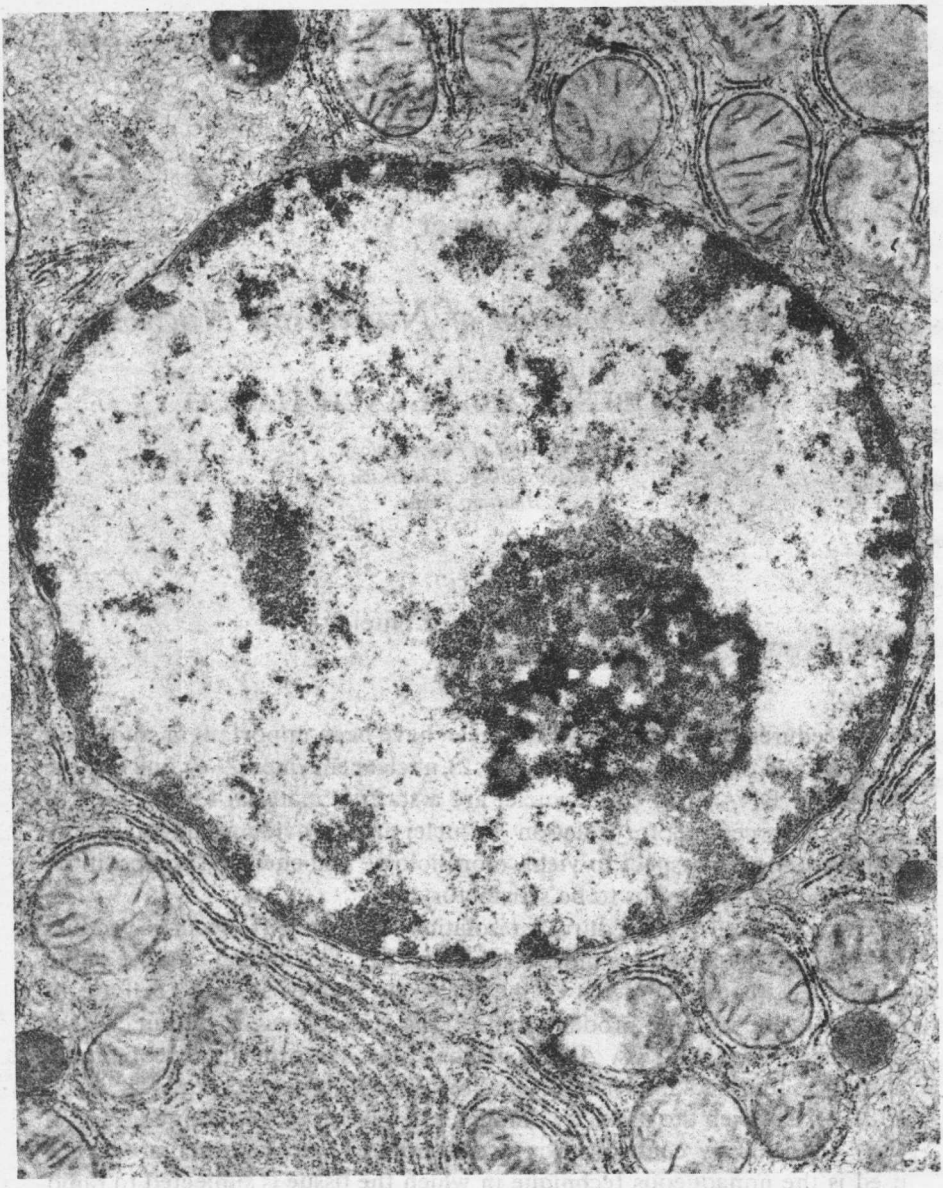

FIG. 1. Electron micrograph of a nucleus within a liver cell showing mitochondria and endoplasmic reticulum in the cytoplasm and the outer layer of the nuclear envelope, which is essentially similar in structure to the endoplasmic reticulum. The inner layer of the nuclear envelope is juxtaposed to dense chromatin masses interspersed between spaces of the nuclear pores. The nucleolar stalk joins the nucleolus to the chromatin at the periphery of the nucleus.