MECHANISMS OF CELL CHANGE

Edited by

JAMES D. EBERT

TOKINDO S. OKADA

MECHANISMS OF CELL CHANGE

Edited by

James D. Ebert
Carnegie Insitution of Washington
Washington, D.C.

Tokindo S. Okada
Kyoto University
Kyoto, Japan

A WILEY MEDICAL PUBLICATION
JOHN WILEY & SONS
New York • Chichester • Brisbane • Toronto

Library of Congress Cataloging in Publication Data:

Main entry under title:

Mechanisms of cell change.

(A Wiley medical publication)
"Based on lectures presented at the symposium entitled Initiation of developmental change, at the VIIIth congress of the International Society of Developmental Biologists held in Tokyo, Japan, August 29–September 2, 1977."
1. Cell differentiation—Congresses. 2. Cellular control mechanisms—Congresses. I. Ebert, James David, 1921– II. Okada, Tokindo, 1927–
III. International Society of Developmental Biologists.
[DNLM: 1. Cytology—Congresses. 2. Growth—Congresses.
QH573 M486 1977]
QL963.5.M4 574.8'761 78-24040
ISBN 0-471-03097-X

Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

MEMBERS OF THE BOARD OF THE INTERNATIONAL SOCIETY OF DEVELOPMENTAL BIOLOGISTS
1977–1981

Michael Abercrombie
Strangeways Research Laboratory
Cambridge, England

Robert DeHaan
Department of Anatomy
Emory University
Atlanta, Georgia

James D. Ebert
Carnegie Institution of Washington
Washington, D.C.

A. Garcia-Bellido
Universidad Autonoma de Madrid
Madrid, Spain

G. Gerisch
Biozentrum der Universitat
Basel, Switzerland

Elizabeth Hay
Harvard Medical School
Boston, Massachusetts

Nicole M. Le Douarin
Institut d'Embryologie du C.N.R.S.
Nogent-sur-Marne, France

V. Monesi
Universita di Roma
Roma, Italy

Alberto Monroy
Zoological Station
Naples, Italy

A. A. Moscona
Department of Biology
University of Chicago
Chicago, Illinois

P. D. Nieuwkoop
Hubrecht Laboratory
Utrecht, Netherlands

Tokindo S. Okada
Department of Biophysics
Laboratory of Developmental Biology
Kyoto University
Kyoto, Japan

D. Osborne
Agricultural Research Council
Cambridge, England

Lauri Saxen
Laboratory of Experimental Embryology
III Department of Pathology
University of Helsinki
Helsinki, Finland

Melvin Spiegel
Department of Biological Sciences
Dartmouth College
Hanover, New Hampshire

Ikuo Takeuchi
Department of Botany
Kyoto University
Kyoto, Japan

A. Tarkowski
University of Warsaw
Warsaw, Poland

S. G. Vassetzky
Institute of Developmental Biology
Moscow, U.S.S.R.

Jorma Wartiovaara
Laboratory of Experimental Embryology
III Department of Pathology
University of Helsinki
Helsinki, Finland

DEDICATION

Louis Gallien
1908–1976

Ernst Hadorn
1902–1976

Reiji Okazaki
1930–1975

It is fitting that this book be dedicated to the memory of three giants in developmental biology and cellular and molecular genetics, whose contributions collectively span virtually the entire range of its chapters. Each was a unique individual, yet they shared many strengths; a profound insight into the forces that shape the developing organism; the rare gifts of critical judgment and rigor that enabled them to select problems because of their importance, not because of their ease; a luminous intensity; and an infectious enthusiasm and willingness to share the joys of discovery with colleagues and students. Their names will be indelibly associated with man's drive to understand the mechanisms of cell change.

Contributors

Mariko Aida
Laboratory of Viral Oncology
Cancer Institute
Tokyo, Japan

Donald D. Brown
Department of Embryology
Carnegie Institution of Washington
Baltimore, Maryland

R. M. Clayton
Institute of Animal Genetics
University of Edinburgh
Edinburgh, United Kingdom

Katsuma Dan
Tokyo Metropolitan University
Tokyo, Japan

Goro Eguchi
Institute of Molecular Biology
Faculty of Science
Nagoya University
Nagoya, Japan

David Epel
Professor of Biological Sciences
Hopkins Marine Station
Department of Biological Sciences
Stanford University
Pacific Grove, California

Günther Gerisch
Department of Cell Biology
Biocenter of the University of Basel
Basel, Switzerland

J. B. Gurdon
M.R.C. Laboratory of Molecular Biology
Cambridge, United Kingdom

Yoshiki Hotta
Department of Physics
Faculty of Science
University of Tokyo
Tokyo, Japan

Yoji Ikawa
Laboratory of Viral Oncology
Cancer Institute
Tokyo, Japan

Yoshio Inoue
Laboratory of Viral Oncology
Cancer Institute
Tokyo, Japan

Reiko Kameji
Laboratory of Viral Oncology
Cancer Institute
Tokyo, Japan

Haruo Kanatani
Department of Developmental Biology
National Institute for Basic Biology
Okazaki, Japan

Takashi Kawachi
National Cancer Research Institute
Tokyo, Japan

Nicole M. Le Douarin
Insitut d'Embryologie du C.N.R.S.
Nogent-sur-Marne, France

Anthony P. Mahowald
Department of Biology
Indiana University
Bloomington, Indiana

Robert E. Maxson
Department of Zoology
University of California
Berkeley, California

Anne McLaren
M.R.C. Mammalian Development Unit
London, United Kingdom

Beatrice Mintz
Institute for Cancer Research
Fox Chase Cancer Center
Philadelphia, Pennsylvania

Masashi Noguchi
Tokyo Metropolitan University
Tokyo, Japan

Masuo Obinata
Laboratory of Viral Oncology
Cancer Institute
Tokyo, Japan

Yoshio Okada
Professor of Animal Virology
Research Institute for Microbial Diseases
Osaka University
Osaka, Japan

Richard E. Pagano
Department of Embryology
Carnegie Institution of Washington
Baltimore, Maryland

Stephen Roth
Department of Biology
The Johns Hopkins University
Baltimore, Maryland

Howard A. Schneiderman
Professor and Director
Center for Pathobiology,
University of California
Irvine, California

Takashi Sugimura
National Cancer Center Research Institute
Tokyo, Japan

Yutaka Uchiyama
Laboratory of Viral Oncology
Cancer Institute
Tokyo, Japan

Isao Uemura
Tokyo Metropolitan University
Tokyo, Japan

Fred H. Wilt
Department of Zoology
University of California
Berkeley, California

Hugh R. Woodland
Department of Biological Sciences
University of Warwick
Coventry, United Kingdom

Preface

John Tyler Bonner's elegant book, *On Development,* illuminates the nature of development by its relation to another theme of biology: evolution. The synthesis of substances in living organisms consists of a series of sequential chemical reactions. As Bonner rightly emphasizes, "Ultimately the information necessary for all these reactions comes from the DNA, but this can be described only in terms of evolutionary development. In the development of a life cycle, there are a series of devices to bring forth and control the stored information. These involve transcription, translation and enzyme activities. They appear in the form of controlled sequences or loops that can be wholly cytoplasmic, involve the genome and the immediate cytoplasm, or, in ever-widening loops, involve inductors, hormones in multicellular organisms, and pheromones in social insects. Many points in these controlled sequences are also influenced by the environment so that the life cycle can accommodate to environmental changes." One of the principal—and common—objectives of developmental biologists today is to understand the mechanisms regulating these sequential reactions.

But development is more than the regulation of synthesis. It is the regulation of the compartmentalization and localization of specific molecules in the cells of developing organisms, by diffusion and active transport, chemical bonding, cell recognition and adhesion, cell migration and growth. In short a temporal sequence of syntheses coupled with mechanisms of compartmentalization produces new patterns of organization. To understand any given developmental state we must understand as well not only the state immediately preceding it but ultimately the evolutionary history of the organism. Thus our emphasis is less on the regulation of synthesis per se than on the mechanisms underlying the transition from one developmental state to another, resulting not only in the appearance of new characters but also in the development of heterogeneity in cell populations leading to the establishment of cell societies.

A concise hypothesis of the mechanism of differentiation was available as early as 1934, when Thomas Hunt Morgan wrote, "It is known that the protoplasm of different parts of the egg is somewhat different, and that the differences become more conspicuous as the cleavage proceeds, owing to the movements of materials that then take place. From the protoplasm are derived the materials for the growth of the chromatin and for the substances manufactured by the genes. The initial differences in the protoplasmic regions may be supposed to affect the activity of the genes. The genes will then in turn affect the protoplasm which will start a new series of reciprocal reactions. In this way we can picture to ourselves the gradual elaboration and differentiation of the various regions of the embryo." Even today this quotation states the essence of our challenge, for its focus is the series of reciprocal reactions impinging upon the cell's inner controls.

In this monograph thirty distinguished contributors explore *the keys to change*, the mechanisms underlying transition in developmental state. At its heart the focus of the book is narrow and deep; the unifying theme is everywhere evident, coursing through the contributions, which embrace an almost bewildering range of changes in state from maturation of the egg, to fertilization, to the rise of diversity of cell types, and to tumorogenesis, in organisms ranging from insects and sea urchins to mice and men. The reader will find diversity in experimental systems and in organisms. The approaches are equally diverse, ranging from biophysics and molecular genetics to cell and tissue morphology to experimental embryology; they should appeal to a wide audience, including immunologists, pathologists and virologists, to name only a few.

Many of the chapters are based on lectures presented at the Symposium entitled "Initiation of Developmental Change" at the VIIIth Congress of the International Society of Developmental Biologists held in Tokyo, Japan, August 29–September 2, 1977. It is our hope that this monograph, like the Symposium, will act as a catalyst in the synthesis of ideas derived from various levels of investigation since as we have said, we wish to reach not only investigators and advanced students in developmental biology, but also biologists in other fields. To that end we added several additional critical and comprehensive chapters, complementing those of the speakers.

The International Society of Developmental Biologists and its Scientific Organizing Committee, which we have been privileged to lead, is deeply indebted to Honorary Co-Chairmen, Professors Katsuma Dan and Takashi Fujii, and Chairman Hiroshi Terayama of the Japanese Organizing Committee, for a remarkably successful Congress, attested to by the genuine enthusiasm of the more than 400 participants. As editors we express our thanks to the contributing authors of this work for their care and uniformly high standards in preparing, and for their promptness in submitting, their manuscripts.

James D. Ebert
Woods Hole, Massachusetts

Tokindo S. Okada
Kyoto, Japan

October 1978

Contents

Part 1

MATURATION AND EARLY DEVELOPMENT

1
Oogenesis: Hormonal Mechanism of Oocyte Maturation

Haruo Kanatani

According to our present understanding of the mechanism of oocyte maturation, some hormones play important roles in inducing breakdown of germinal vesicles and subsequent meiotic maturation. In vertebrates it has long been known that pituitary gonadotropins control growth and ripeness of the gonads. However, with respect to oocyte maturation, it was only in 1967 that some specific hormones, such as progesterone, were found to induce oocyte maturation in amphibians (1, 2). Pituitary gonadotropins stimulate the follicles around the oocytes to produce or secrete progesterone or a progesterone-like hormone, which induces oocyte maturation. In the same year (1967), in invertebrates a similar hormonal mechanism of oocyte maturation was found in starfish by Schuetz and Biggers (3) in Woods Hole and by Kanatani and Shirai (4) at the Ocean Research Institute. Since then several studies have elucidated the hormonal mechanism of oocyte maturation in starfishes as well as in amphibians (5, 6). In teleosts, accumulated evidence also suggests that a similar hormonal mechanism induces oocyte maturation, with progesterone and cortisol being the responsible hormones (7, 56).

In this chapter I present a survey focused largely on studies of the starfish and report the recent findings of our laboratory, since the hormonal mechanism of oocyte maturation in the starfish appears to be fully representative of the general phenomenon.

GONAD-STIMULATING SUBSTANCE

It was not that long ago that a hormonal substance related to reproduction was first found in echinoderms. In 1959 Chaet and McConnaughy (8, 9) found that a water extract of the radial nerves of *Asterias forbesi* induced shedding of gametes when injected into a ripe starfish (Fig. 1–1). Additional studies by several investigators (5) reveal that the active substance contained in the nerve extract is comparable to

The research for this study was supported in part by grants-in-aid from the Ministry of Education, Science and Culture and the Ford Foundation

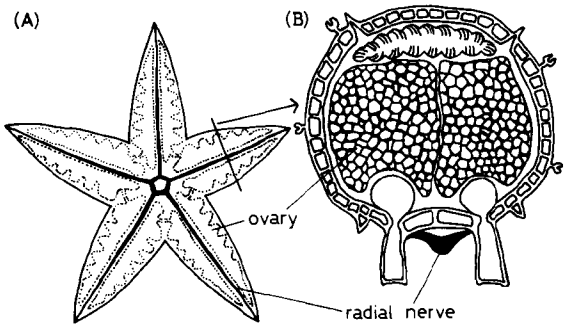

Figure 1–1. (*a*) Starfish, showing sites of radial nerves and ovaries. (*b*) Cross section of arm.

gonadotropins in vertebrates; it has been called gonad-stimulating substance (GSS) (10), or radial nerve factor (RNF) (11).

When isolated ovarian or testis fragments are kept in seawater containing the nerve extract, they begin to discharge their gametes from the cut surface after about 30 minutes (11–13). When ovarian fragments are ligated to prevent the discharge of eggs and treated with GSS-seawater for one hour, histological sections clearly show that the oocytes within the ovary are undergoing the first maturation division, whereas the germinal vesicles of oocytes in untreated ovaries remain intact. In starfishes, oocytes within a ripe ovary remain at the late prophase stage of meiosis. Therefore GSS was once thought to induce oocyte maturation as well as spawning (11, 14–16). Electron microscopic observations on the radial nerves and on the granules containing GSS isolated with differential centrifugation and sucrose-density gradient ultracentrifugation from the homogenate of the radial nerves reveal that GSS is present in the granules contained in the supporting cells, which are located at the base of the radial nerve (17).

Concerning its chemical nature, GSS of *Asterias amurensis* was purified and identified as a heat-stable polypeptide, with a molecular weight of about 2100 estimated by the ultracentrifugal analysis (18), consisting of the following amino acids: aspartic acid (number of residues are 2), threonine (1), serine (6), glutamic acid (1), proline (1), glycine (4), alanine (2), valine (1), isoleucine (1), leucine (1), histidine (1) and ornithine (1). Since GSS does not contain cysteine or cystine, it seems to be a simple chain-like molecule. Molecular weight of *Asterias* GSS determined by a simple gel-filtration on Sephadex G-50 column was between 2000 and 2300, indicating that the GSS molecule does not decompose during the purification. The isoelectric point of *Asterias* GSS is at about pH 4.5. The purified GSS induced shedding of oocytes from the isolated ovarian fragments at concentration of 0.0096 μg/ml (18).

MATURATION-INDUCING SUBSTANCE

At the time of starfish spawning, GSS comparable to vertebrate gonadotropin is secreted from the nervous tissue, most likely from the supporting cells in the radial nerves, into the coelomic cavity where the gonads are suspended (19, 20). This hormone, which by itself fails to induce maturation of isolated oocytes, acts on the follicle cells in the ovary or specific interstitial cells in the testis. It stimulates these cells to produce and secrete a maturation-inducing substance, MIS (3, 4, 21–23). The MIS of *Asterias amurensis* was

purified from the supernatant of artificial seawater containing the ovarian fragments cultured for six hours with GSS and chemically identified as 1-methyladenine (1-MeAde) (24, 25). If GSS is considered to be the primary hormone in starfish oocyte maturation, 1-MeAde is the second hormone that acts directly on the oocytes as a trigger of maturation. It is comparable to progesterone or progesterone-like substances in amphibians and to progesterone or cortisol in teleosts.

When the isolated oocytes with their follicular envelopes are placed in seawater containing 1-MeAde, they undergo germinal vesicle breakdown and subsequent meiotic maturation (26, 27). The follicular envelopes are also broken down at the same time (28). Although the effective dose of 1-MeAde varies according to the reactivity of the oocytes, it lies at around 3×10^{-7} to 10^{-8} M (26, 29, 30). Discharge of oocytes from the ovary is also induced by treatment with 1-MeAde *in vivo* as well as *in vitro* (26).

1-MeAde brings about some morphological changes on the surface of the oocyte (21, 31). An electron microscopic study revealed that the vitelline coat of non-nucleated oocytes as well as of intact oocytes of *Asterina pectinifera* became flat and thin after treatment with 1-MeAde. Also there were fewer microvilli of the oocyte surface protruding into the vitelline coat. Probably they are withdrawn to the surface of the oocyte. The elevation of the fertilization membrane is observed upon insemination of oocytes from which the germinal vesicle has been removed, and which has been treated with 1-MeAde. On the other hand, without 1-MeAde treatment immature starfish oocytes fail to form the fertilization membrane upon insemination. These facts strongly suggest that cytoplasmic maturation, at least at the surface of the starfish oocyte, can be induced by 1-MeAde without any participation of germinal vesicle material. In other words, acquisition of fertilizability of the egg cytoplasm is independent of the contribution of the germinal vesicle to oocyte maturation (21, 31).

Elevation of the fertilization membrane from the immature oocyte of *Asterias* and *Asterina* upon insemination can be induced by pretreating the oocyte with calcium-free seawater (32, 33). In this case, however, polyspermy occurs. On the other hand, 1-MeAde-treated oocytes block polyspermy upon insemination. In *Pisaster giganteus*, Lee et al (34) reported that the oocytes elevated the fertilization membrane upon insemination without previous induction of maturation by 1-MeAde or germinal vesicle breakdown. Abnormal development considered to be due to polyspermy was observed. Hence 1-MeAde seems to act on the oocyte surface to establish the polyspermy block mechanism, although details of this activity are still unknown (33).

Further, concerning other actions of 1-MeAde, Shirai (35) has recently shown that the site of polar body formation on starfish oocyte is shifted to some extent toward the site of local application of 1-MeAde. Her method consisted in sucking up a fully grown oocyte of *Asterina pectinifera* in the tip of a capillary tube so as to locate its germinal vesicle at the center of the oocyte. Then she locally stained one side of it with nile blue and the opposite side with neutral red. When these oocytes were placed in seawater containing 1-MeAde, polar bodies were formed equally in both the nile blue and neutral red hemispheres. Hoever, when 1-MeAde was locally applied to the nile blue-stained region, most of the polar bodies were formed in the nile blue-stained hemisphere. This result indicates that 1-MeAde affects the site of polar body formation.

Biochemical Mechanism and Activity

To understand the biochemical mechanisms underlying the biological activity of 1-MeAde, we searched for a group of substances, of which both the chemical action is

known and the biological activity is similar to that of 1-MeAde. Along this line, Kishimoto and Kanatani (36) found that disulfide-reducing agents—such as dithiothreitol (DTT) and 2,3-dimercapto-1-propanol (BAL)—induce oocyte maturation. When isolated oocytes of *Asterina pectinifera* were placed in seawater containing DTT at concentrations around 10^{-2} M, they underwent 100% maturation. On the other hand, pretreatment with sulfhydryl reagents such as p-chloromercury benzoate (PCMB), iodoacetamide and N-ethylmaleimide completely suppressed 1-MeAde–induced oocyte maturation. This inhibition of sulfhydryl reagents on oocyte maturation was diminished by subsequent treatment with DTT or BAL, regardless of the presence of 1-MeAde.

Oocyte maturation induced by DTT is similar to that induced by 1-MeAde with respect to the morphological changes and the time of the successive events. The next experiment was to determine if the reduction of protein–disulfide bonds in the cortical region of the oocyte occurs in 1-MeAde-induced maturation. Cortices of *Asterias* oocytes treated with 1-MeAde for different intervals were obtained and the amount of protein-derived sulfhydryl (protein-SH) of these cortices was determined (37). The sulfhydryl content of oocyte–cortex protein first increased after 1-MeAde administration and then decreased. These oocytes underwent the breakdown of germinal vesicle within 15–25 minutes after treatment with 1-MeAde.

The amount of protein-SH in the cortex increased and reached a maximum before the breakdown of germinal vesicle. Further, a chronological correlation was found between the maximal increase in SH content and the start of breakdown of germinal vesicle, i.e., the shorter the time to reach the maximal level of SH content, the earlier the breakdown of germinal vesicle. This fact indicates that the reduction of disulfide-bond in this region is involved in the early step of oocytic maturation that is required for the subsequent events of this phenomena. Possibly the effect of 1-MeAde in inducing starfish oocyte maturation is ascribable to its disulfide-reducing action, although the underlying mechanism is still unknown.

In addition to the increase in sulfhydryl content of oocyte–cortex protein, Guerrier et al (38) recently reported that protein kinase activity of whole oocyte homogenate of *Marthasterias glacialis* increases five minutes after administration of 1-MeAde. Further, they showed that, following the addition of 1-MeAde, protein phosphorylation is stimulated *in vivo* and *in vitro* in both the cortical and endoplasmic regions of *Marthasterias* oocytes (39). Moreau et al (40) extended this study to amphibian oocyte maturation and found that injection of beef-heart protein kinase or rabbit phosphorylase kinase into *Xenopus laevis* oocytes brings about their maturation. Further, Maller and Krebs (41) found that progesterone-stimulated oocyte maturation in *Xenopus* can be inhibited by microinjection of the catalytic subunit of adenosine 3′,5′-monophosphate–dependent protein kinase from muscle and directly induced in the absence of progesterone after microinjection of its regulatory subunit.

MATURATION-PROMOTING FACTOR

In oocyte maturation, 1-MeAde acts on the oocyte surface. Injection of 1-MeAde into the oocytes with germinal vesicles fails to induce their maturation, but oocytes placed in a dilute 1-MeAde solution of less than about 10^{7} M undergo breakdown of germinal vesicles within 20 minutes, and meiotic maturation follows (42).