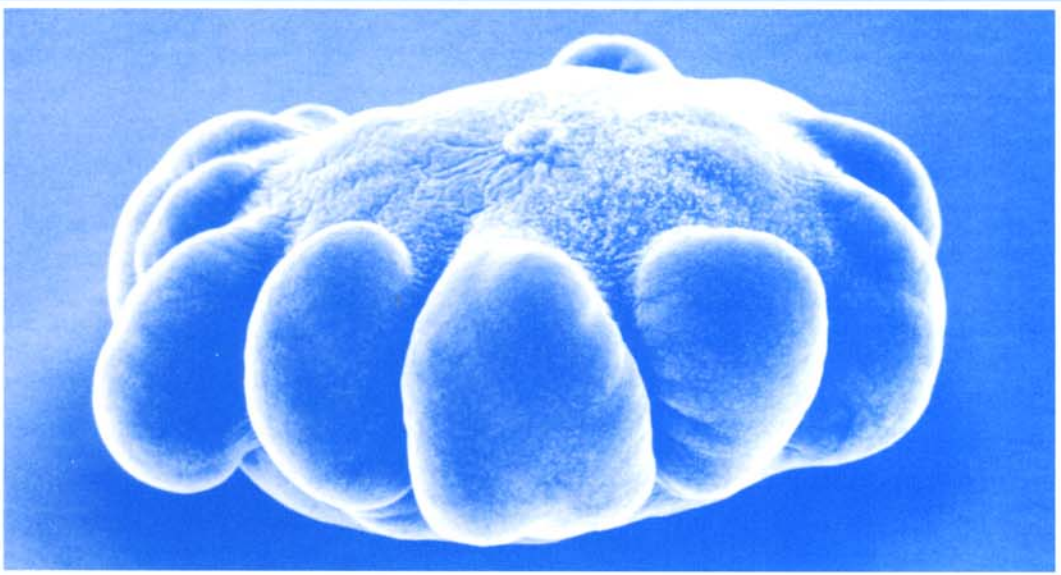
Cytoskeletal Mechanisms during Animal Development

Edited by

David G. Capco



Current Topics in Developmental Biology, Volume 31

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Developmental Biology

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Cytoskeletal Mechanisms during Animal Development

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Edited by

David G. Capco
Molecular and Cellular Biology Program
Arizona State University
Tempe, Arizona

Academic Press
San Diego New York Boston London Sydney Tokyo Toronto

Front cover photograph: Scanning electron micrograph showing the side view of an egg undergoing the deformation movement. (For more details see Chapter 7, Figure 6.)

This book is printed on acid-free paper. ♾

Academic Press, Inc.
A Division of Harcourt Brace & Company
525 B Street, Suite 1900, San Diego, California 92101-4495

United Kingdom Edition published by
Academic Press Limited
24-28 Oval Road, London NW1 7DX

International Standard Serial Number: 0070-2153

International Standard Book Number: 0-12-153131-7

PRINTED IN THE UNITED STATES OF AMERICA
95 96 97 98 99 00 BC 9 8 7 6 5 4 3 2 1

Contents

3

Sea Urchin Microtubules

Kathy A. Suprenant and Melissa A. Foltz Daggett

4

Actin–Membrane Cytoskeletal Dynamics in Early Sea Urchin Development

Edward M. Bonder and Douglas J. Fishkind

5

RNA Localization and the Cytoskeleton in *Drosophila* Oocytes

Nancy Jo Pokrywka

8

Development and Evolution of an Egg Cytoskeletal Domain in Ascidians

William R. Jeffery

9

Remodeling of the Specialized Intermediate Filament Network in Mammalian Eggs and Embryos during Development: Regulation by Protein Kinase C and Protein Kinase M

G. Ian Gallicano and David G. Capco

10

Mammalian Model Systems for Exploring Cytoskeletal Dynamics during Fertilization

Christopher S. Navara, Gwo-Jang Wu, Calvin Simerly, and Gerald Schatten

11

Cytoskeleton in Teleost Eggs and Early Embryos: Contributions to Cytoarchitecture and Motile Events

Nathan H. Hart and Richard A. Fluck

12

Confocal Immunofluorescence Microscopy of Microtubules, Microtubule-Associated Proteins, and Microtubule-Organizing Centers during Amphibian Oogenesis and Early Development

David L. Gard, Byeong Jik Cha, and Marianne M. Schroeder

13

Cortical Cytoskeleton of the *Xenopus* Oocyte, Egg, and Early Embryo

Carolyn A. Larabell

14

Intermediate Filament Organization, Reorganization, and Function in the Clawed Frog *Xenopus*

Michael W. Klymkowsky

Contributors

Numbers in parentheses indicate the pages on which the authors' contributions begin.

Winston A. Anderson Department of Biology, Howard University, Washington, District of Columbia 20059; and Marine Biological Laboratory, Woods Hole, Massachusetts 02543 (5)

Edward M. Bonder Department of Biological Sciences, Rutgers University, Newark, New Jersey 07102; e-mail: bonder@actin.rutgers.edu (101)

David G. Capco Molecular and Cellular Biology Program, Arizona State University, Tempe, Arizona 85287; e-mail: atdgc@asuvm.inre.asu.edu (277)

Danièle Carré Unité de Biologie Cellulaire Marine, (URA 671 CNRS/ Université Paris VI), Observatoire de Villefranche-sur-mer, Station Zoologique 06230 Villefranche-sur-mer, France (41)

Byeong Jik Cha Department of Biology, University of Utah, Salt Lake City, Utah 84112 (383)

Patrick Chang Unité de Biologie Cellulaire Marine, (URA 671 CNRS/ Université Paris VI), Observatoire de Villefranche-sur-mer, Station Zoologique, 06230 Villefranche-sur-mer, France;
e-mail: chang@ccrv.obsvlfr.fr (41)

William R. Eckberg Department of Biology, Howard University, Washington, District of Columbia 20059; and Marine Biology Laboratory, Woods Hole, Massachusetts 02543; e-mail: eckberg@access.howard.edu (5)

Douglas J. Fishkind Department of Biological Sciences, University of Notre Dame, Notre Dame, Indiana 46556; e-mail: douglas.j.fishkindi@nd.edu (101)

Richard A. Fluck Department of Biology, Franklin & Marshall College, Lancaster, Pennsylvania 17604; e-mail: r_fluck@acad.fandom.edu (343)

Melissa A. Foltz Daggett Department of Physiology and Cell Biology, University of Kansas, Lawrence, Kansas 66045 (65)

G. Ian Gallicano Molecular and Cellular Biology Program, Arizona State University, Tempe, Arizona 85287 (277)

David L. Gard Department of Biology, University of Utah, Salt Lake City, Utah 84112; e-mail: gard@bioscience.utah.edu (383)

Nathan H. Hart Department of Biological Sciences, Rutgers University, Piscataway, New Jersey 08855 (343)

Evelyn Houliston Unité de Biologie Cellulaire Marine, (URA 671 CNRS/ Université Paris VI), Observatoire de Villefranche-sur-mer, Station Zoo-

logique, 06230 Villefranche-sur-mer, France;
e-mail: houliston@ccrv.obsvlfr.fr (41)

William R. Jeffery Section of Molecular and Cellular Biology, University of California, Davis, California 95616; e-mail: wrjeffery@ucdavis.edu (243)

Michael W. Klymkowsky Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, Colorado 80309; e-mail: klym@spot.colorado.edu (455)

Carolyn A. Larabell Lawrence Berkeley National Laboratory, University of California, Berkeley, California 94720; e-mail: larabell@lbl.gov (433)

Kathryn G. Miller Department of Biology, Washington University, St. Louis, Missouri 63130; e-mail: miller@biodec.wustl.edu (167)

Christopher S. Navara Departments of Zoology and Obstetrics and Gynecology and the Wisconsin Regional Primate Research Center, University of Wisconsin, Madison, Madison, Wisconsin 53706;
e-mail: csnavara@students.wisc.edu (321)

Nancy Jo Pokrywka Department of Biology, Vassar College, Poughkeepsie, New York 12601; e-mail: napokrywka@vaxsar.vassar.edu (139)

Christian Sardet Unité de Biologie Cellulaire Marine, (URA 671 CNRS/ Université Paris VI), Observatoire de Villefranche-sur-mer, Station Zoologique, 06230 Villefranche-sur-mer, France;
e-mail: sardet@ccrv.obsvlfr.fr (41)

Gerald Schatten Departments of Zoology and Obstetrics and Gynecology and the Wisconsin Regional Primate Research Center, University of Wisconsin, Madison, Madison, Wisconsin 53706;
e-mail: schatten@macc.wisc.edu (321)

Marianne M. Schroeder Department of Biology, University of Utah, Salt Lake City, Utah 84112 (383)

Takashi Shimizu Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060, Japan;
e-mail: shitak@clu.hines.hokudai.ac.jp (197)

Calvin Simerly Departments of Zoology and Obstetrics and Gynecology and the Wisconsin Regional Primate Research Center, University of Wisconsin, Madison, Madison, Wisconsin 53706;
e-mail: csimerly@macc.wisc.edu (321)

Kathy A. Suprenant Department of Physiology and Cell Biology, University of Kansas, Lawrence, Kansas 66045;
e-mail: ksupre@kuttub.cc.ukans.edu (65)

Gwo-Jang Wu[1] Departments of Zoology and Obstetrics and Gynecology and the Wisconsin Regional Primate Research Center, University of Wisconsin, Madison, Madison, Wisconsin 53706 (321)

[1] Present Address: Department of Ob/Gyn, TSGH, Taipei, Taiwan 100, Republic of China.

Notice

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Introduction

Gametes, zygotes, and blastomeres of the embryo are cells and must exhibit all of the functional characteristics of a cell in order to survive. In addition to all the requisite cell functions, gametes, zygotes, and blastomeres of the embryo face challenges posed by the developmental program that regulates these cells. Gametes, zygotes, and embryos contain adaptations that allow these specialized cells to meet and surmount the challenges posed by the developmental program. These developmental challenges are directed at the structure and function of these specialized cells, and consequently the adaptations act through specializations in the cytoskeleton.

Many of these specializations in the cytoskeleton are most clearly detectable at the time that these specialized cells undergo major remodeling of structure and function, that is, at the time of a developmental transition. Developmental transitions represent major partitions or landmarks in the developmental program where the gametes, zygote, or blastomeres of the embryo undergo a major structural and functional change. Several developmental transitions are common to (or conserved among) all classes of organisms, for example, gametogenesis, fertilization, and gastrulation. In addition, there are typically developmental transitions specialized for classes of organisms, for example, see Chapters 5, 6, 9, and 10. These transitions cause a radical change in cell function due to an underlying remodeling of intracellular structure (or in the case of the multicellular embryo both intracellular and intercellular remodeling result). This remodeling alters the engineering of the cell, and as a consequence, the function of the cell changes.

The chapters in this volume focus on the cytoskeletal specializations that allow these cells to face and surmount the special developmental problems unique to gametes, zygotes, and blastomeres of the embryo. In each of the chapters readers will identify specializations of the cytoskeleton to meet the challenges of the developmental program that exist at both conserved and specialized developmental transitions. These cytoskeletal specializations set gametes, zygotes, and blastomeres of the embryo apart from somatic cells and also demonstrate remarkable adaptability in elements of the cytoskeleton and in the elaboration of cytoskeletal structures.

Much of the current understanding of cytoskeletal organization and function comes from analysis of results obtained from studies of somatic cells.

The somatic cells employed in many of these studies were obtained either from cell lines maintained *in vitro* (e.g., 3T3 mouse fibroblasts, MDCK cells, endothelial cell) or by explant from the organism (e.g., blood platelets, macrophages, intestinal epithelium). From studies on such cells a minimum of four roles for the cytoskeleton are generally accepted: (1) The cytoskeleton provides the shape and infrastructural support for a cell as well as positioning the organelles and nucleus. (2) Elements of the cytoskeleton serve as "roadways" for the movement of cellular components, including membranous elements, through the action of molecular motors. (3) The cytoskeleton also positions both proteins and mRNA in nonrandom distributions within cells, presumably at sites where such components are necessary. (4) The cytoskeleton mediates cell motility.

The somatic cell types used to obtain the information outlined in the previous paragraph are certainly important and central to the field of cell biology. However, it must be recognized that there are limitations to the type of knowledge obtained by analysis of somatic cells that can be applied to the understanding of cells exhibiting specialized developmental roles. These limitations exist at two levels. First, not all cells will survive under *in vitro* culture conditions, and most that do lose their histotype. Even those cells that are explanted from an organism and studied immediately, such as intestinal epithelial cells, may retain their histotype, but may exhibit a wound response that modifies the action of the cytoskeleton. Thus, while results obtained from investigation of such cells certainly represent an activity of the cytoskeleton within the cell's repertoire, they may not representative of the activity of the cell in its natural location or normal histotype. Moreover, they may not be representative of cell types that cannot be maintained for *in vitro* analysis even for short-term studies. Second, these somatic cells do not face the special developmental challenges of gametes, zygotes, and blastomeres of the embryo.

What are the special developmental challenges faced by gametes, zygotes, and blastomeres and what adaptations exist to allow these special cells to overcome the challenges? The answer to that question is the subject of this volume. Some of these challenges will be common to all species, whereas other challenges will be species-specific. The chapters in this volume present these aspects for several classes of organisms. Any developmental biologist could easily conceive of some of the challenges presented by the developmental program that are conserved among different classes of organisms. A few examples follow: (1) Oocytes, eggs, and blastomeres of the early embryo contain an unusually large cytoplasmic volume compared to that of somatic cells. This can present special problems in intracellular communication when the cell must undergo a coordinated change, such as a progression through the cell cycle in the case of blastomeres or a response of the egg to the penetrating sperm. (2) The zygote is developmentally totipotent

through the elaboration of its developmental program. No somatic animal cell is developmentally totipotent. (3) Fertilization requires cell fusion (i.e., between the egg and the sperm). In most species a mechanism exists to permit entry of only one sperm. Typically, somatic cells do not fuse (this statement excludes the terminal expression of a developmental program in cell types such as muscle). Even when somatic cells are induced to fuse through experimental manipulation, for example, to produce a hybridoma, a totipotent zygote is not produced. (4) Fertilization requires the restoration of ploidy through the unification of two different populations of chromosomes without the loss of a chromosome or part of a chromosome. This event occurs as pronuclear fusion or the unification of the two chromosomal populations during M phase of the cell cycle. Fusion of somatic cells through experimental manipulations usually results in the loss of one or more chromosomes from the heterokaryon. (5) Eggs and blastomeres of embryos exhibit unusual cell cycle regulation (i.e., specific cell cycle arrest points for eggs and modified cell cycles for blastomeres). Typically, a somatic cell is either progressing through the cell cycle (i.e., a stem cell) as is the case for skin epithelial cells, or it is arrested late in Gap_1 of the cell cycle in a state referred to Gap_0. In the latter case, the cell cycle arrest point differs from that of the egg, as does the mechanism of recusing the cell from Gap (e.g., the cell cycle arrest in the egg is released by fusion with the sperm). In the former case where the stem cell is progressing through the cell cycle, the amount of time spent in Gap_1, Gap_2, and the synthesis phase (DNA synthesis) for the stem cell is significantly longer than the times exhibited by blastomeres of the embryo.

Several of the conserved modifications of cytoskeletal function that have been identified in eggs, zygotes, and blastomeres address some of these developmental challenges. Some examples follow: (1) To allow for rapid, synchronized changes in large cells, such as the egg, cytoplasmic signal transduction mechanisms are responsible for the rapid remodeling events (of all parts of the egg including the cytoskeleton) at the developmental transition that converts the egg into the zygote. (2) Where examined, microtubule arrays appear to participate in the approximation of male and female pronuclei within the egg/zygote cytoplasm, permitting syngamy to occur. (3) Eggs contain extensive, cortical cytoskeletal domains that remodel as a result of fertilization and perhaps permit exocytosis of cortical granules, which provides the long-term block to polyspermy. (4) In those cases investigated, the cortical cytoskeletal domain has been shown to be associated (in some cases directly and in other cases indirectly) with components capable of influencing the developmental fate of subsequently formed blastomeres. (5) Developmental transitions are accompanied by a remodeling of both the cortical and the internal cytoskeletal components, and in those

cases investigated the cytoskeletal remodeling has been shown to be regulated by cytoplasmic signal transduction mechanisms.

The occurrences outlined in the previous paragraph, and other developmental roles for the cytoskeleton, are presented in more detail in this volume. The studies in this volume demonstrate a central role for the cytoskeleton in development. Moreover, these studies demonstrate that the cytoskeleton in eggs, zygotes, and blastomeres of the embryo is a remarkably malleable structure. Even more remarkable is that the three main filament networks (i.e., networks composed of actin filaments, microtubules, and intermediate filaments) are capable of this vast array of specialized activities. To date, no new filament network has been identified in association with these special cellular functions during development, although the existing cytoskeletal networks have been identified in highly unusual aggregations and forms.

The cytoskeleton exhibits functions and activities in these specialized cells that, to date, have no parallels in somatic cells. Yet all somatic cells ultimately arise from the penetration of an egg by a sperm. Could it be that these specialized activities of the cytoskeleton are involved only during development and that once a somatic cell is formed the cytoskeleton no longer can exhibit these special roles? Or could it be that our knowledge of cytoskeletal function in somatic cells is skewed by the cell types available to cell biologists for study? Let us look and wonder together.

Preface to Section I: Nonchordates

The first section of this volume focuses on cytoskeletal mechanisms involved with early development in nonchordates. The chapters listed in parentheses denote chapters in which comparable cytoskeletal mechanisms also have been reported.

In Chapter 1 (by William Eckberg and Winston Anderson) evidence is presented to demonstrate cytoskeletal involvement with cell shape changes that accompany fertilization, and also both the localization of mRNA and the redistribution of mRNAs into specific patterns within the zygote (Chapters 3, 5, 8, and 12–14). In addition, this chapter demonstrates that the egg contains an extensive cortical cytoskeleton (referred to as cortical cytoskeletal domain), while the cytoskeleton in the egg interior is highly reduced. This chapter also considers mechanisms for regulation of cytoskeletal organization by the action of signal transduction events, specifically the level of intracellular free calcium and the action of protein kinase C (Chapters 3, 4, 7, 9, 11, 13, and 14).

Chapter 2 (by Evelyn Houliston and co-workers) examines cytoskeletal mechanisms in ctenophore development. Again, the cytoskeleton is involved in physical changes in cell shape. In addition, evidence is presented which suggests involvement of microtubules in promotion of pronuclear juxtaposition (Chapters 3 and 10) as well as in cortical rotation (similar to that found during insect oogenesis; Chapter 5) and postfertilization development (Chapters 8, 12, and 13) in some chordates that promotes axis formation. Moreover, the cytoskeleton is involved in positioning of morphogenetic determinants (Chapters 1, 5, and 7).

Two chapters examine cytoskeletal mechanisms in sea urchin eggs. The microtubule network in sea urchin eggs is the focus of Chapter 3 (by Kathy Suprenant and Melissa Foltz). Here the authors present a role for microtubules in pronuclear movement (Chapters 2 and 10) and discuss a special cortical cytoskeletal domain composed of microtubules. They review the data indicating that the dynamics of microtubule assembly is regulated by signal transducers such as kinases and phosphatases (Chapters 1, 4, 5, 7, 9, 11, and 14). Finally, they present evidence that ribosomes attach to the cytoskeleton (whereas in other systems reports demonstrate mRNA attached to the cytoskeleton; Chapters 1, 3, 5, 8, and 12–14) and that this may have a role in translational regulation. In Chapter 4 (by Bonder and

Fishkind) the actin cytoskeleton of the sea urchin egg is examined. Here evidence is presented to demonstrate that the cortical actin cytoskeleton undergoes extensive remodeling at the time of fertilization (Chapters 1, 2, 7, 10, 12, and 13), that the egg undergoes a cortical contraction (Chapters 1, 2, 7, and 13), and that signal transduction events regulate the reorganization of the cytoskeleton (Chapters 3, 7, 9, 11, and 14).

There are two chapters on insect development, specifically *Drosophila.* Both chapters highlight the ability to conduct experiments which manipulate the genetics of the system to reveal functional roles for the filament systems as well as for a variety of cytoskeleton-associated proteins. The first, Chapter 5 (by Nancy Jo Pokrywka), considers the involvement of the cytoskeleton during oogenesis. This chapter reviews the evidence for very distinct roles for the actin filament network and the microtubule network in translocating material between the nurse cells and the oocyte. It hints at a role for the action of kinases to regulate the microtubule network (Chapters 1, 3, 4, 7, 9, 11, and 14) and demonstrates a role for microtubules in the positioning of specific RNAs and in the repositioning of the RNA as development ensues (Chapters 1, 3, 8, and 12–14). However, here the evidence suggests a two-phase process is involved in positioning of RNA (i.e., an initial localization followed by stabilization; Chapter 12). Evidence also is considered that suggests that microtubules may be involved in the rotation of the cortical cytoplasm (referred to as ooplasmic streaming), which is somewhat similar to the cortical rotations described in other systems (Chapters 2, 8, and 12); however, here the streaming occurs prior to fertilization, whereas in the other systems it occurs after fertilization. Chapter 6 (by Kathryn Miller) focuses on the role of the actin cytoskeleton in postfertilization development. In this chapter, data are considered that present a role for actin in establishing cytoplasmic domains surrounding each embryonic nucleus. The development of this embryo as a syncytium for the first nine nuclear division cycles presents special problems in regulating chromosomal separation. The actin network serves as a highly regulated mechanism (over space and time) to isolate the genetic material into distinct cytoplasmic islands. This chapter presents a variety of mutants that are certain to provide an understanding of specific functions for the cytoskeleton.

The final chapter in this section, Chapter 7 (by Takashi Shimizu), details cytoskeletal mechanisms in early development of the freshwater oligochaete, *Tubifex.* Here evidence is presented which suggests that the actin network is involved in the shape change of the egg, and that the actin network forms a cortical cytoskeletal domain. This cortical-actin domain contracts into distinct subdomains (Chapters 1, 2, 4, 7, 12, and 13) and influences the movement of morphogenetic determinants (Chapters 1, 2, 5, and 7). A role for a specific kinase in the remodeling of the cytoskeletal network is considered (Chapters 1, 3–5, 7, 9, 11, 13, and 14). In addition, a role of centrosomal positioning in unequal cleavage divisions is considered.

Section I

Cytoskeletal Mechanisms in Nonchordate Development

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Cytoskeleton, Cellular Signals, and Cytoplasmic Localization in *Chaetopterus* Embryos

William R. Eckberg and Winston A. Anderson
Department of Biology
Howard University
Washington, DC 20059
and
Marine Biological Laboratory
Woods Hole, Massachusetts 02543

I. Introduction

A. *Chaetopterus* as a Model System in Developmental Biology

The use of *Chaetopterus* as a system for study in developmental biology evidently began with E. B. Wilson (1882), who briefly described its early development along with that of several other annelids. Experimental analysis of the development of this organism began with Jacques Loeb (1901)

Current Topics in Developmental Biology, Vol. 31

in a paper in which he misdescribed "differentiation without cleavage" (Lillie, 1902) resulting from K^+ activation as true parthenogenesis. Other early studies on cytoplasmic localization and embryo organization in relation to development were performed by such legendary developmental and cellular biologists as F. R. Lillie (1906, 1909), E. B. Wilson (1929, 1930), T. H. Morgan (1910, 1937, 1938, 1939; Whitaker and Morgan, 1930; Morgan and Tyler, 1938), A. Tyler (Titlebaum, 1928; Tyler, 1930), E. B. Harvey (1939), J. Brachet (1937, 1938, and more recent work cited under Section II,C), and J. Pasteels (1935, 1950).

Experimental studies of the development of *Chaetopterus* have emphasized three areas: the regulation of the cell cycle (germinal vesicle breakdown, GVBD), mechanisms of fertilization and egg activation, and the effects of egg organization on development. Although this chapter will stress the last area, we will briefly mention the advantages of *Chaetopterus* oocytes and eggs for the first two areas. The primary advantages for the study of GVBD are that large numbers of oocytes ($>10^6$ cells or 2 ml) can be obtained from a female at one time, and that all can be induced to undergo GVBD synchronously in response to either their natural trigger (an unknown trace component in seawater) or to certain cellular agonists/antagonists of known biological activity. A further advantage is that the oocytes then arrest at metaphase I of meiosis until fertilized or artifically activated. In other words, the cells can be induced to undergo the G_2/M phase transition at will without continuing to cycle. Furthermore, these oocytes can be easily labeled with isotopic markers. The availability of large numbers of synchronized, easily labeled eggs is also an important consideration in studies of fertilization and egg organization. The unique advantages of *Chaetopterus* in studies of fertilization are that the fertilizing sperm interact with morphologically definable structures on the egg surface (Anderson and Eckberg, 1983) and that the egg is at least as metabolically active before fertilization as it is after. In fact, the unfertilized egg uses much more O_2 than does the fertilized (Whitaker, 1933) or artifically activated (Brachet, 1938) egg. The fact that the physiology of the initiation of development in *Chaetopterus* eggs differs from that of sea urchins should make them an object of more detailed study.

The unique feature which makes *Chaetopterus* of particular interest for studies of egg and embryo organization in development is the ability of the artifically activated or fertilized egg to undergo differentiation without cleavage (Lillie, 1902). Artificial activation can be induced by excess KCl (Lillie, 1902; Brachet, 1937). Differentiation without cleavage also occurs in fertilized eggs subjected to temporary cleavage inhibition by treatment with cytochalasin B (Eckberg, 1981a) and in polyspermic embryos. This interesting phenomenon will be discussed in greater detail under Section II,C.

B. Early Development

Fertilized eggs undergo typical spiral cleavage. The first five cleavages are synchronous; thereafter, cleavage becomes highly asynchronous. While the cell lineage has not been followed as completely in this organism as that in some other spiralians, there is no reason to expect that it differs significantly in *Chaetopterus* (*cf.* Henry and Martindale, 1987).

The first cleavage is unequal due in part to the presence of a small polar lobe and more importantly to asymmetric placement of the metaphase spindle. In some embryos, polar lobes do not form, but the cleavage is still unequal and subsequent development is normal. Polar lobes typically have substantial morphogenetic significance as shown by the fact that removal of the polar lobe results in severely deficient embryos, although this is less true for *Chaetoperus* than for most other lobe-bearing spiralians that have been studied.

Early investigations suggested that unequal cleavage distributes morphogenetic substances unequally to the two blastomeres (Titlebaum, 1928; Tyler, 1930). Equalization of the first cleavage by compression resulted in the development of embryos with duplication of many structures. In blastomere isolation studies of *Chaetopterus,* as in similar studies on other spiralian species, isolated AB cells formed a "swimming mass, mainly of ectodermal cells," whereas isolated CD cells developed into structures which "outwardly resembled" early trochophores and occasionally formed advanced trochophores. From this description, however, the possibility that many (or all) of these "embryos" had actually undergone differentiation without cleavage cannot be excluded, because swimming masses or structures which outwardly resemble trochophores can develop in this species in the complete absence of cell division.

In fact, the results of more recent studies suggest that *Chaetopterus* embryos differ from those of other lobe-bearing spiralians in that removal of the polar lobe has only a marginal effect on larval morphogenesis (Henry, 1986). In this study, lobeless embryos cleaved normally and formed larvae which were normal except that they lacked the ability to produce bioluminescence. Even AB and CD blastomeres developed similarly to each other and to control embryos in most respects. Further studies on equalized cleavage in these embryos (Henry and Martindale, 1987) provided a wide range of results from complete symmetric twinning through incomplete twinning to normal embryos. Normal embryos accounted for about half of the cases studied. The finding that many equally cleaving embyos developed normally is unusual for spiralia, but consistent with the results of blastomere isolation experiments. In agreement with these results, other studies in which the first cell division was equalized by another method resulted in development of embryos that were normal with no apparent doubling of

posterior structures (Eckberg, 1981a). In this study, cytochalasin B (CB) was used to block first cleavage. The drug was then washed out of the embryos, which then proceded to cleave into either two or four equal-sized cells at the time controls cleaved into four cells. These last results must be interpreted with some caution, however, as the embryos were not followed to advanced stages, and CB has dramatic effects on embryonic cytoskeleton organization (see below).

II. Cytoplasmic Localization

A. Localizing Movements in Living Eggs

Chaetopterus eggs undergo a series of dramatic changes in cell shape prior to first cleavage (Fig. 1). The spherical zygote flattens slightly along the animal/vegetal axis coincidently with the formation of each polar body (Fig. 1A). About 15 min after second polar body formation, the egg becomes constricted at the animal pole into a "pear" shape (Fig. 1B). Within 5 min the elongation disappears and the cell becomes flattened at the animal pole. Coincidently with this, a constriction occurs near the vegetal pole

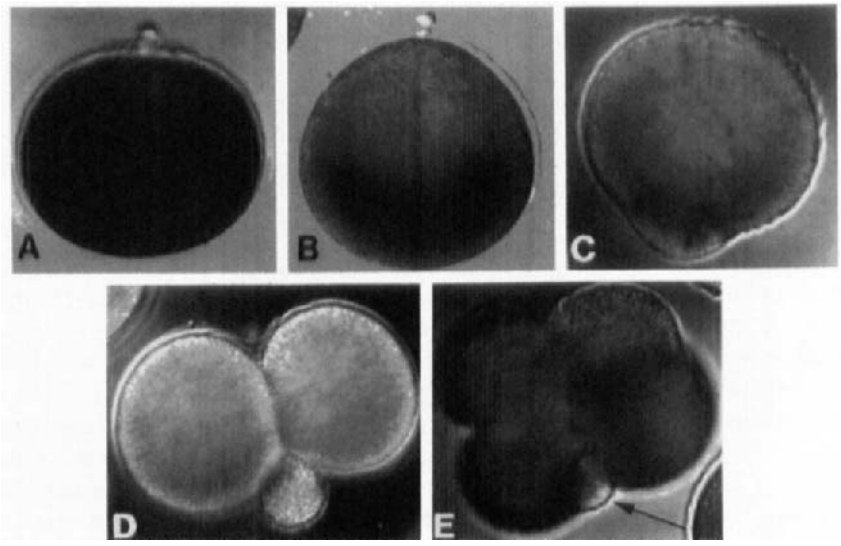

Fig. 1 Cell shape changes in living *Chaetopterus* embryos prior to first cleavage and during formation of the polar lobes. (A) Embryo flattened during second polar body formation, (B) embryo elongated ("pear-shaped") during mitosis, (C) initial formation of the first polar lobe during anaphase, (D) definitive polar lobe during first cleavage (the CD cell is on the left), and (E) second polar lobe (arrow) during second cleavage. ×320.