Ultrastructure of Reproduction

J. Van Blerkom and P. M. Motta, editors

Martinus Nijhoff Publishers

Ultrastructure of Reproduction

Gametogenesis, Fertilization, and Embryogenesis

Edited by

Jonathan Van Blerkom, Ph.D.
Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado, USA

and

Pietro M. Motta, M.D., Ph. D.
Department of Anatomy, Faculty of Medicine, University of Rome, Rome, Italy

1984 **MARTINUS NIJHOFF PUBLISHERS**
a member of the KLUWER ACADEMIC PUBLISHERS GROUP
BOSTON / THE HAGUE / DORDRECHT / LANCASTER

Distributors

for the United States and Canada: Kluwer Boston, Inc., 190 Old Derby Street, Hingham, MA 02042, USA
for all other countries: Kluwer Academic Publishers Group, Distribution Center, P.O. Box 322, 3300 AH Dordrecht, The Netherlands

Library of Congress Cataloging in Publication Data

Main entry under title:

Ultrastructure of reproduction.

(Electron mioroscopy in biology and medicine ; v. 2)
Includes index.
1. Gametogenesis. 2. Embryology--Vertebrates.
3. Fertilization (Biology) 4. Ultrastructure (Biology)
I. Van Blerkom, Jonathan, 1947- . II. Motta,
Pietro. III. Series. [DNLM: 1. Germ cells--
Ultrastructure. 2. Embryo--Ultrastructure. 3. Fertilization. 4. Microscopy, Electron--Methods. WQ
205 U47]
QL964.U38 1983 599'.32 83-2437
ISBN 0-89838-572-5

Copyright

PRINTED IN THE NETHERLANDS

Ultrastructure of reproduction

ELECTRON MICROSCOPY IN BIOLOGY AND MEDICINE

Current Topics in Ultrastructural Research

SERIES EDITOR: P.M. MOTTA

Already published in this series:

Motta, P.M. (ed.): Ultrastructure of Endocrine Cells and Tissues.
ISBN: 0-89838-568-7

Preface

Advances in the development and application of electron microscopic techniques have occurred recently such that the electron microscope has evolved to become an essential tool in both basic and clinical research. Use of this instrument has contributed significantly to the formation of new perspectives and concepts concerning cell fine structure. These structural perspectives are now being integrated with specific functional, biochemical and pathophysiological events and processes of cells and tissues.

Most recently, utilization of innovative electron microscopic techniques such as freeze-fracture, freeze-etching, and scanning and high-voltage electron microscopy offers both the basic and clinical scientist potentially fundamental insights into many morphodynamic processes related to the activities of cells and tissues. Such an approach has been especially rewarding when applied to the dynamic events of gametogenesis and early embryonic development.

The chapters comprising this book have been selected and edited with the aim of providing an up-to-date and comprehensive account of the most important aspects of vertebrate gamets and embryos as revealed by the integration of several different submicroscopic methods. The organization of the chapters is designed to indicate present gaps in our knowledge of the developmental and reproductive biology of gametes and the developing embryo and possible lines of research which may lead to a lessening of these gaps.

The contributions to this volume have been prepared by experts in the field. Each chapter has been composed with the intent of offering the reader not only a concise history of the topic but also emphasizing research problems that must be solved in the future. Much of the information has been presented in an illustrative format, because only in this way will it be apparent how the integration of ultrastructural and biochemical techniques contribute to a more comprehensive understanding of gametogenesis and embryonic development.

This volume should prove useful to advanced students of reproductive and developmental biology, researchers and clinicians. It will be of specific interest to developmental, cell and reproductive biologists involved in the research and teaching of embryology, histology and physiology in both veterinary and medical schools.

We express our thanks to the many authors, not only for the quality of their contributions but also for their patience in responding to exacting and often tedious editorial demands. Our grateful appreciation also is extended to Mr. Hobart Bell for his insightful and very helpful suggestions in the preparation of this book.

October, 1983

Jonathan Van Blerkom, Boulder, Colorado
Pietro M. Motta, Rome, Italy

List of contributors

Azevedo, Carlos. Department of Cell Biology, Institute of Biomedical Sciences, University of Oporto, 4000, Oporto, Portugal

Baccetti, Baccio. Institute of Zoology, University of Siena, Via Mattioli 4, 53100 Siena, Italy

Baker, Terry G. School of Medical Sciences, University of Bradford, Bradford, BD7 1DP, United Kingdom

Chávez, Daniel J. Department of Anatomy, School of Medicine, Southern Illinois University, Carbondale, IL 62901, USA

Coimbra, Antonio. Institute of Histology and Embryology, Faculty of Medicine, University of Oporto, 4200, Oporto, Portugal

Dvořák, Milan. Department of Histology and Embryology, J.E. Purkyně University, tř. Obráncu miru 10, 662 43 Brno, Czechoslovakia

Eddy, Edward M. Department of Biological Structure, University of Washington School of Medicine, Seattle, WA 98195, USA

Franchi, Leslie L. Department of Anatomy, University of Birmingham, Birmingham, B15 2TJ, United Kingdom

Friend, Daniel S. Department of Pathology, University of California School of Medicine, San Francisco, CA 94143, USA

Fujimoto, Toyoaki. Department of Anatomy, Kumamoto University Medical School, Kumamoto, 860, Japan

Gondos, Bernard. Department of Pathology, University of Connecticut Health Center, Farmington, CT 06032, USA

Goodall, H. Department of Anatomy, Cambridge University, Cambridge, CB2 3DY, United Kingdom

Handyside, A. Department of Anatomy, Cambridge University, Cambridge, CB2 3DY, United Kingdom

Hoffman, Loren H. Department of Anatomy, Vanderbilt University, Nashville, TN 37232, USA

Johnson, M.H. Department of Anatomy, Cambridge University, Cambridge, CB2 3DY, United Kingdom

Kaye, Jerome S. Department of Biology, University of Rochester, Rochester, NY 14627, USA

Kopečný, Václav. Department of Histology and Embryology, J.E. Purkyně University, tř Obráncu miru 10, 662 43 Brno, Czechoslovakia

Mc Gaughey, Robert W. Department of Zoology, Arizona State University, Tempe, AZ 85287, USA

Merchant-Larios, Horacio. Instituto de Investigaciones Biomédicas, Universitad Nacional Autónoma de

México, Apartado Postal 70228, Ciudad Universitaria 04510 Mexico, D.F., Mexico

Nilsson, Ove. Department of Human Anatomy, University of Uppsala, Biomedical Center, Uppsala, Sweden

Olson, Gary E. Department of Anatomy, Vanderbilt University, Nashville, TN 37232, USA

Phillips, David M. The Population Council, Center for Biomedical Research, 1230 York Avenue, New York, NY 10021, USA

Plöen, Leif. Department of Anatomy and Histology, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, S-75007, Uppsala, Sweden

Pratt, H.P.M. Department of Anatomy, Cambridge University, Cambridge, CB2 3DY, United Kingdom

Reeve, W.J.D. Department of Anatomy, Cambridge University, Cambridge, CB2 3DY, United Kingdom

Ritzén, Martin. Pediatric Endocrinology Unit, Karolinska Sjukhuset, S-10401, Stockholm, Sweden

Russell, Lonnie D. Department of Physiology, School of Medicine, Southern Illinois University, Carbondale, IL 62901, USA

Szöllösi, Daniel. Station centrale de Physiologie Animale, I.N.R.A. 78350 Jouy-en-Josas, France

Tesařík, J. Department of Histology and Embryology, J.E. Purkyně University, tř. Obráncu miru 10, 662 43 Brno, Czechoslovakia

Ukeshima, Atsumi. The College of Medical Science, Kumamoto University, Kumamoto 862, Japan

Wiley, Lynn M. Department of Human Anatomy, University of California, Davis, CA 95616, USA

Yotsuyanagi, Yoshio. Centre de Génétique Moléculaire, C.N.R.S. 91190 Gif-sur-Yvette, France

Ziomek, C.A. The Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545, USA

Contents

CHAPTER 1

Origin of the germ cell line

EDWARD. M. EDDY

1. Introduction

The definitive germ cell line usually is considered to begin with the appearance of primordial germ cells (PGCs). As we shall see, for a few selected examples, PGCs are identified first in the posterior blastoderm in certain insect embryos, in the floor of the blastocoel in anuran frogs, in the anterior extraembryonic endoderm in chicks and in the endoderm of the yolk sac in mammals. It is tempting to make the generalization from these findings that the germ cell line of diverse animals arises within the endoderm or its equivalent prior to organogenesis. However, PGCs usually are identified on the basis of morphological features. This criterion alone may be insufficient for defining the beginning of the germ cell line. Cells which do not particularly look like PGCs but which have the developmental potential of PGCs, or express gene products specific to PGCs, might be present at earlier times and at other sites during embryogenesis. If this were shown to be the case, one might argue that by these criteria such cells should be called PGCs. One of the main problems, then, in discussing the origin of the germ cell line is in defining a germ cell, particularly one at an early point in the lineage. Is there a marker or a set of markers that can be used to reliably identify precursors of PGCs before they acquire the overt morphological characteristics of germ cells? As will be seen, the answer is yes for some species, but no for others. For the latter species, the question then becomes: Are there markers which have not yet been identified or are there no markers because there is no germ cell line before PGCs can be recognized? There have been several reviews on various aspects of the origin of the germ cell line in different organisms in the last few years (1–5). This Chapter takes a comparative approach but with a focus on mammals, considering in particular the site of origin of the germ cell line and the possible role of germ plasm in germ line determination.

2. Animals with a continuous germ line

Certain nematodes have been found to have determinative cleavage with the egg dividing asymmetrically four times, each time giving rise to a larger somatic tissue precursor cell and to a smaller P cell. At this point, the P_4 cell divides, giving rise to two germ cell line precursor cells (6, 7). It has been found recently that some lots of rabbit antisera identify cytoplasmic granules within cells of the germ cell lineage of *Caenorhabditis elegans* (8). At the first cleavage, the granules are located at the posterior end of the embryo where the P cells form. During the next three cleavages, the granules lie at the side of the cell where the next P cell will form and through this process the granules become segregated into the single precursor cell for the germ cell line. The granules are similar in distribution and size to specific structures previously observed by electron microscopy in some P cells and in subsequent germ cells (9). In other studies, exudation of substantial amounts of cytoplasm from different regions of the uncleaved egg, after puncturing the egg capsule with a laser beam, seldom resulted in sterile embryos, indicating that the putative determinants for the germ cell line were probably not present in the cytoplasm of uncleaved eggs in an unbound form (10). However, neither the cytoplasm that was lost not that remaining was examined for the presence of the granules characteristic of cells in the germ cell lineage.

It was realized by early investigators that the pole cells which bud off the posterior tip of certain insect embryos prior to formation of the cellular blastoderm give rise to PGCs (e.g., 11). Some of the features of pole cells in *Drosophila* are that they are derived clonally from 8–10 nuclei, they divide 1–3 times in the early embryo but then arrest until after gastrulation, they interdigitate within the blastoderm prior to gastrulation with some reaching the boundary of the yolk, and they are loosely attached to the

Van Blerkom, J. and Motta, P.M. (eds.), Ultrastructure of reproduction. ISBN 0-89838-572-5.

midgut rudiment during gastrulation (12). Shortly after gastrulation, most pole cells migrate to the lateral mesoderm where the gonads form and those pole cells which remain outside the gonads degenerate (13).

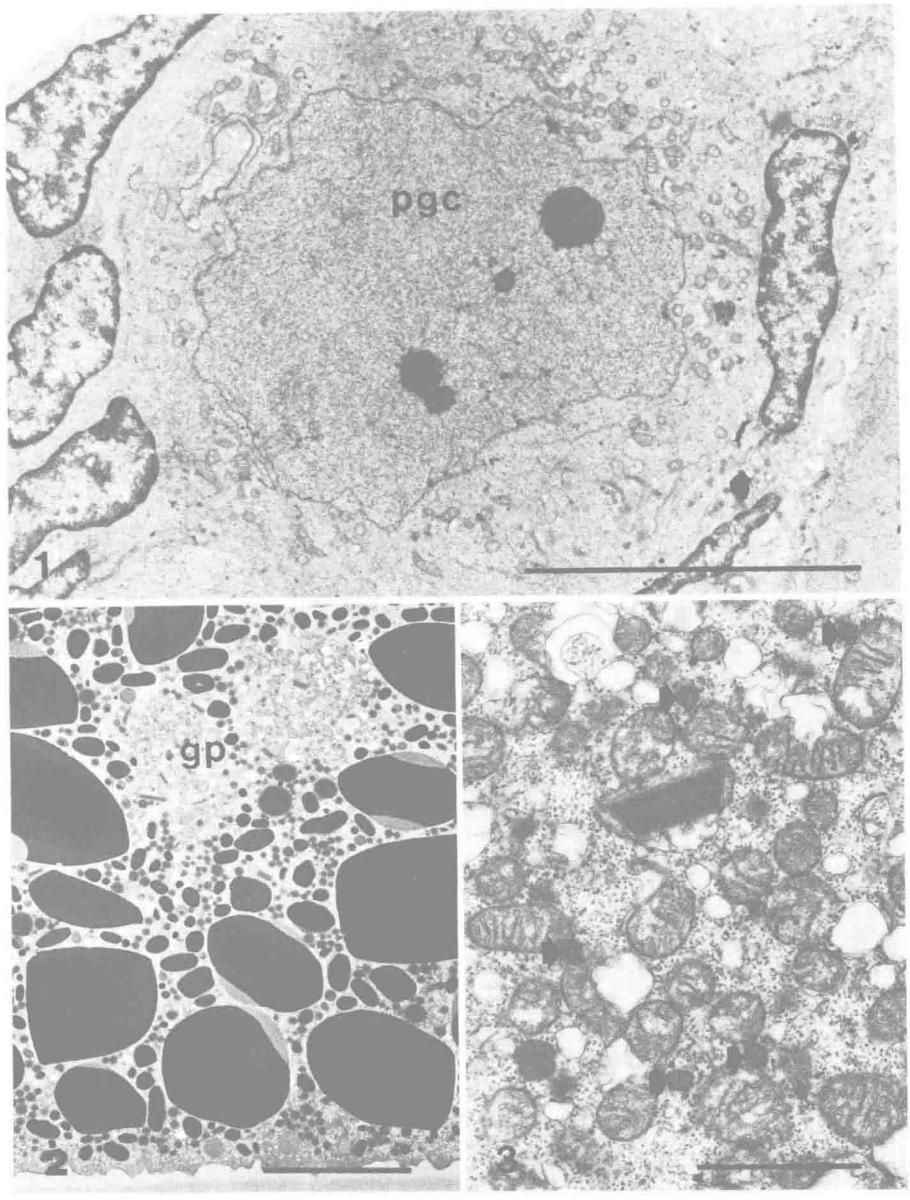
A characteristic feature of pole cells in *Drosophila* and some other insects is the presence of 'polar granules' in the cytoplasm. A number of morphological studies, initially at the light microscope level (14), and more recently using electron microscopy (e.g., 15), have traced the polar granules from the posterior cytoplasm of the oocyte or mature egg to the pole cells and subsequently into definitive germ cells. Because of this location, Hegner proposed that the polar granules are either germ cell determinants or the visible sign of the germ cell determinant region (14). This hypothesis has been supported repeatedly by results of experimental studies. Surgical ablation of the posterior cytoplasm (16), ultraviolet irradiation of the posterior tip of the mature egg (17, 18), or displacement of polar granules by centrifugation (19), all result in insects lacking germ cells. More recently, it has been shown that transplantation of posterior cytoplasm from donor eggs to eggs exposed to ultraviolet irradiation restores the ability of the treated eggs to form germ cells (20). In other experiments, posterior egg cytoplasm transplanted to the anterior tip of the embryo resulted in cells there which contained polar granules. When those cells were grafted in turn to the posterior pole of other embryos, some of the progeny of the resulting adults bore genetic markers from the donor embryos, showing that posterior cytoplasm can cause anterior blastoderm cells to become PGCs capable of forming gametes (21). A subcellular fraction enriched for polar granules has been examined by SDS polyacrylamide gel electrophoresis and found to contain a major basic protein with a molecular weight of approximately 95,000 that was not present in other cells (22). Additional studies are needed to determine the role of this protein in polar granule structure and function.

In anuran amphibians, cells having the appearance and behavior of PGCs are present in the endoderm of neurula stage embryos. In larval stage embryos, these cells move to the dorsal endoderm and migrate through the dorsal mesentery to enter the genital ridges (Fig. 1) (23). These cells contain distinctive cytoplasmic granules which have been traced from uncleaved, fertilized eggs of *Rana temporaria* where they are aligned along the plasma membrane at the vegetal pole, into a few of the vegetal blastomeres during cleavage (Fig. 2), subsequently to cells in the presumptive endoderm forming the floor of the blastocoele during gastrulation and finally into the definitive PGCs. Bounoure termed these granules the *cytoplasme germinal* (germ plasm), and suggested that they were responsible for determining the germ cell line (23). Similar observations have been made since in other species of amphibians (reviewed in 2, 24). Furthermore, fine structural studies (Fig. 3) have established that the cytoplasmic granules consist of aggregates of fibrogranular material lacking a surrounding membrane (e.g., 25).

Ablation and restoration experiments have given further evidence that the germ cell line is determined by factors from the region of the amphibian egg and early embryo containing the germ plasm. It has been shown that surgical removal of the vegetal pole cytoplasm from eggs, 2-cell or 4-cell embryos results in animals with few or no germ cells (26, 27), with the amount of germ plasm that remains determining the number of PGCs that form (28). It was also shown that irradiation of the vegetal pole of fertilized eggs with ultraviolet light also results in sterility in frogs which develop from treated eggs (29). This effect was found to be dose-dependent and stage-dependent (30) and to vary between batches of eggs (31). The most convincing evidence came when Smith (30) injected subcortical cytoplasm from the vegetal pole of unirradiated eggs (about 3% of the total egg volume) into the same region of eggs which had been irradiated and found that 47% of the injected eggs developed into larvae containing PGCs. Since larvae which developed from irradiated eggs that either were uninjected or injected with animal pole cytoplasm lacked PGCs, it seems likely that vegetal pole cytoplasm, containing germ plasm is responsible for determining PGC formation in these embryos. Thus, for animals from three different phyla there appears to be a marker for the germ cell line which can be traced from the fertilized egg to definitive germ cells. Furthermore, there is experimental evidence in *Drosophila* and in anuran amphibians that the marker is either the determinant of the germ cell line or is at least spatially associated with the determinant.

3. Animals not shown to have a continuous germ line

In consideration of some animals in which a continuous germ cell lineage has not been demonstrated, there are conflicting reports and opinions on the origin of PGC in urodelean amphibians. They are less thoroughly studied than anurans, and although germ plasm is apparently present in fertilized *Ambystoma* eggs (31) and PGCs (32), the germ plasm has not been traced from eggs through early development



Figs. 1–3. (1) A PGC in the genital ridge of a *Rana pipiens* tadpole is recognizable as a large cell with an irregularly shaped nucleus containing round, dense nucleoli and sparse heterochromatin. Scale line equals 10 μm. (2) A region of the vegetal cytoplasm of a 2-cell stage *Rana pipiens* embryo is shown. Yolk granules of various sizes are present surrounding regions of cytoplasm containing germ plasm (GP). Scale line equals 10 μm. (3) An area from Figure 2 is shown at higher magnification. The fibrogranular germ plasm is indicated by arrows. Scale line equals 1 μm. (Figs. 2 and 3 reprinted by permission (2)).

to PGCs. Some investigators have concluded from morphological or experimental studies that PGCs have an endodermal origin in urodeles similar to that in anurans (33–35). Other investigators have concluded that a germ plasm is not involved in determination of PGCs in urodeles, but rather that they arise epigenetically from totipotent cells in the mesoderm under the inductive influence of the endoderm (3). Although the experimental evidence is consistent with the latter hypothesis, it does not exclude the possibility of an endodermal origin as in anurans. Additional studies clearly will need to be carried out in urodeles to determine if there is a developmental continuity of germ plasm and if it has a role in the determination of the germ cell line.

In the chick, PGCs are first recognized in the hypoblast layer of the extraembryonic endoderm in the region of the germinal crescent (36). They appear

there at the head process stage, around 18 hr after fertilization, when the primitive streak has formed but before somites are present (37). The PGCs are rich in PAS-positive material (38), being more abundant in later stages than during earlier times or during PGC migration (39). As development proceeds the PGCs accumulate at the junction of the ectoderm and endoderm and enter the blood islands when the mesoderm invaginates to the germinal crescent area (40). Following this, the PGCs enter the circulation, are transported to the gonadal precursors and then leave the circulation to colonize the gonads (41). There is as yet no morphological or experimental evidence of a germ plasm associated with germ cell formation in the chick.

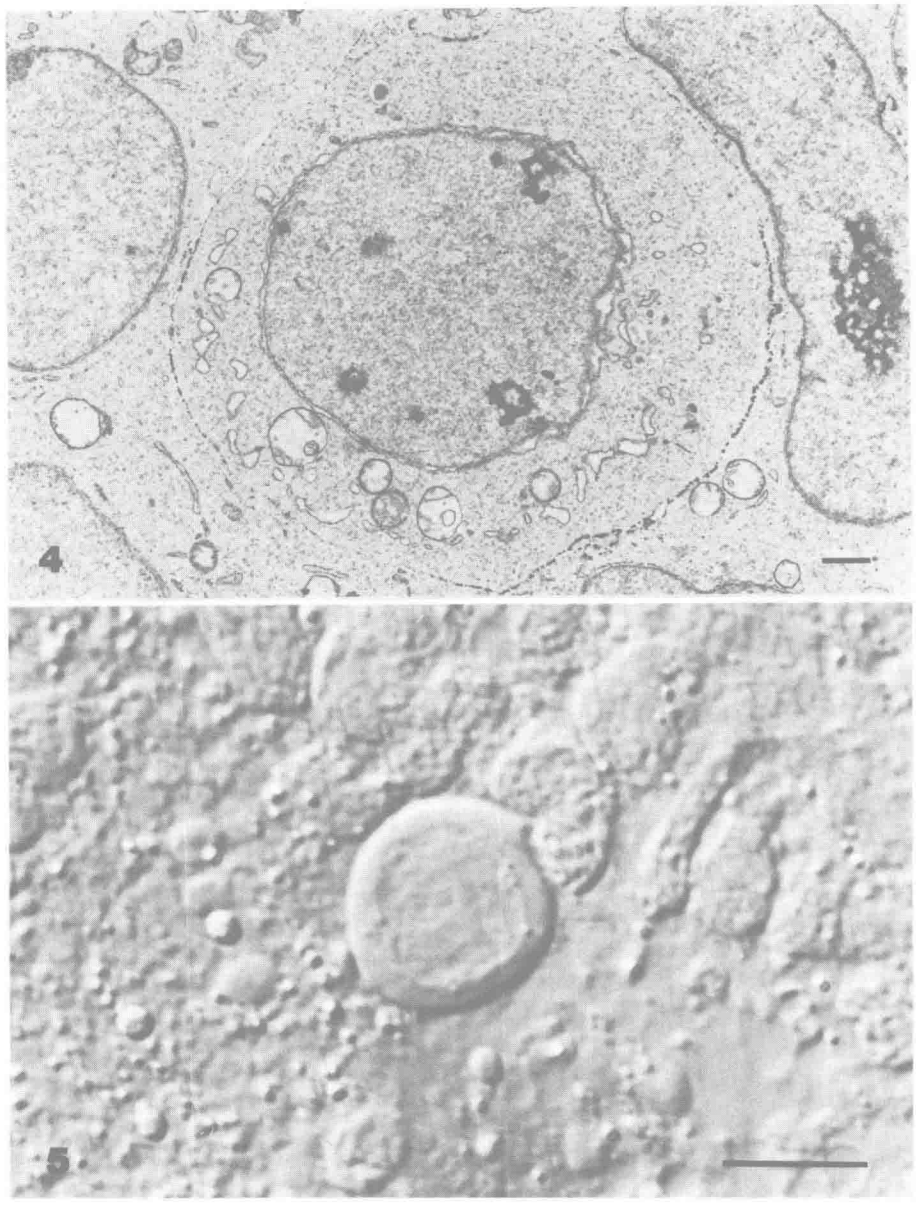
The time and site of origin of PGC precursors in the chick, as opposed to when PGCs are first recognized, has not yet been well established. Different segments of the blastoderm have been taken from chick embryos and incubated through the time period corresponding to when PGCs are first recognized. One study indicated that PGC precursors originate in the primary hypoblast of the posterior region of the blastoderm and are transported anteriorly to the germinal crescent by morphogenetic movements (42). However, another study found that the usual number of PGCs could arise in anterior halves of the blastoderm as well as in posterior halves (43). Similar results have been obtained from studies using even earlier stages, before blastoderm formation (44), suggesting that PGC formation in the chick may occur in the preblastoderm period throughout the region destined to become extraembryonic endoderm. However, x-irradiation of either the anterior or posterior half of the blastoderm prior to incubation leads to a reduction in the number of PGCs, while x-irradiation of the posterior half after a few hours of incubation causes only a slight reduction in number of PGCs. It has been suggested that presumptive PGCs may be distributed throughout the endoderm during the preblastoderm and early blastoderm stages, but accumulate in the anterior half during the first few hours of incubation upon segregation of the primary hypoblast from the epiblast (44).

Early workers debated whether the germ cell line in mammals arose in the gonads or was of extragonadal origin (45). Morphological evidence for the extragonadal origin was gained with the finding that PGCs could be distinguished from surrounding somatic cells by their high alkaline phosphatase activity (Fig. 4) (46). It was soon demonstrated that PGCs could be found in the vicinity of the yolk sac endoderm and the root of the allantois on the 8th day of gestation in the mouse at about the time somites first appear (47–49). In addition to the presence of alkaline phosphatase on their surface, PGCs in mammals are recognizable as being large, round cells (Fig. 5) with blunt pseudopodia and containing abundant free ribosomes (Fig. 6) but relatively few other organelles (50–52). There is also good experimental evidence that PGCs have an exclusive extragonadal origin in mammals and are not supplemented by cells arising in the gonads. When genital ridges from mouse embryos are transplanted to beneath the kidney capsule in adults before germ cells appeared, they fail to develop germ cells even though other structures develop, including mesonephric tubules, mesonephric ducts and Müllerian ducts (53). Also mutations which result in sterility in homozygotes have been found to result in embryos with normal numbers of extragonadal PGCs in early embryos but with few PGCs in the gonads at later stages (48). These and other morphological and experimental studies clearly indicate that PGCs in mammals have an extragonadal origin prior to the appearance of the gonadal anlagen and that they migrate to the gonads at a later stage in development (see Merchant, Chapter 3).

4. Nuage and germ cells

In addition to those animals in which a visible marker has been traced from the egg cytoplasm through cells of the early embryo to lie in definitive germ cells, a larger number of animals have been reported to contain structures morphologically similar to the germ line markers in their germ cells (2). The term 'nuage' (54) has been applied to these structures. Although some of the reports came from light microscopic studies, most of the observations occurred after the advent of electron microscopy. Nuage usually is found to be a discrete, dense, fibrous cytoplasmic organelle which lacks a surrounding membrane and often lies in association with mitochondria (Fig. 7) or adjacent to nuclear pores. Although nuage is strikingly similar in form and distribution to polar granules of *Drosophila* and germ plasm of amphibians, neither nuage nor the germ line markers have been sufficiently well characterized biochemically or functionally to determine if they are related.

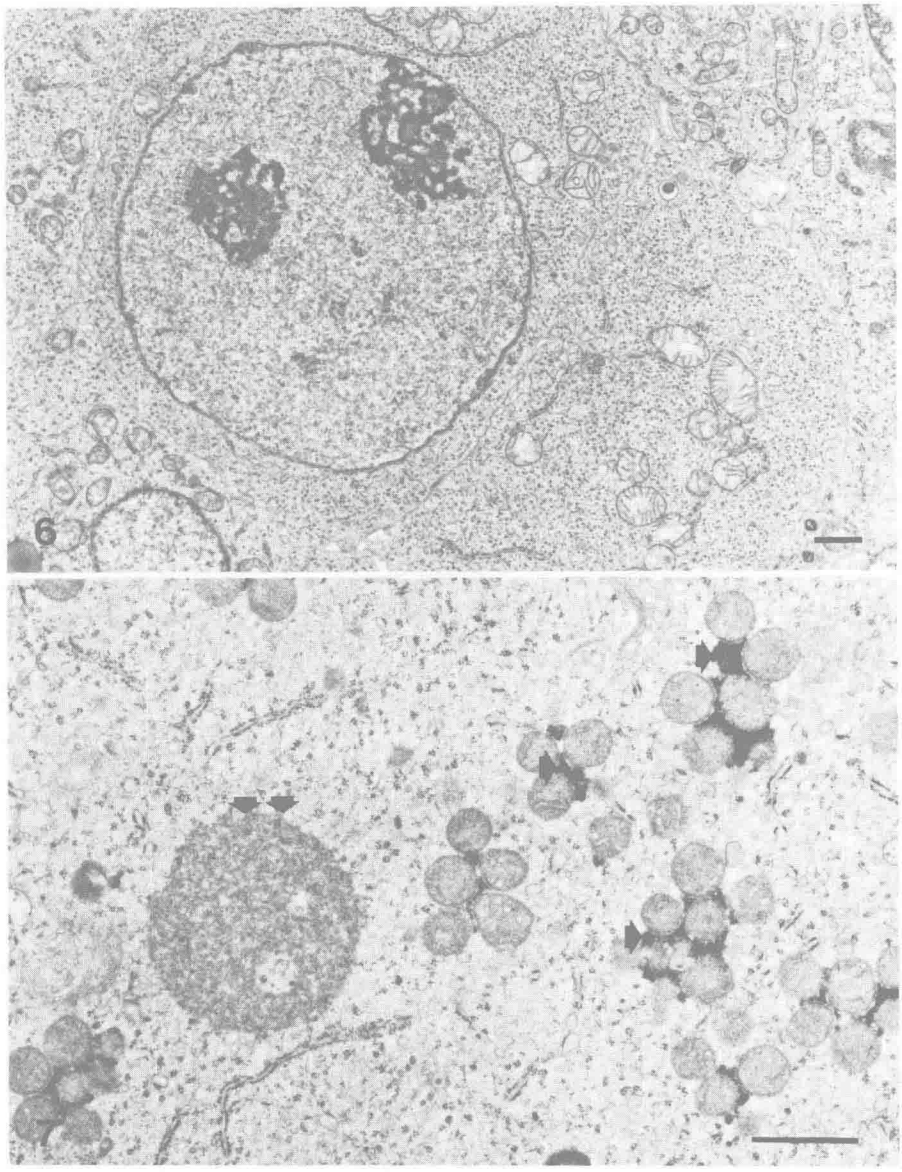
The earliest point in the development of the germ cell line in mammals that nuage has been reported is in PGCs in the hindgut epithelium of 9-day mouse embryos (51) and of 10-day rat embryos (55, 56). Nuage is present in the subsequent stages of germ cell formation in both males and females, including



Figs. 4–5. (4) A PGC in the genital ridge of a 14-day rat embryo that has been stained for alkaline phosphatase. The histochemical reaction product is deposited along the plasma membrane of the PGC. Scale line equals 1 μm. (5) A living PGC is seen by Nomarski optics in a squash preparation of a genital ridge from a 12-day rat embryo. PGCs are recognizable at the light microscope level because of their large size, rounded profile and usual solitary distribution. Scale line equals 10 μm.

oocytes, in several mammalian species (reviewed in 2). It is particularly abundant in some species such as the golden hamster (57), but unfortunately is rather sparse in the mouse, the favorite animal for studying early mammalian development. Several ultrastructural studies on cleavage and blastulation in this species did not report nuage (e.g., 58–59). However, these studies were not specifically intended for detecting nuage and it is possible that by using a different species in which nuage is more prominent or by using selective probes that recognize nuage, it might be possible to trace nuage throughout early embryogenesis in mammals. Indeed, there is one report containing convincing electron micrographs of nuage in early stages of embryogenesis of the rabbit (60).

Clearly, it is not possible either to exclude or to implicate nuage in the determination of the germ cell line in mammals based on the limited information presently available. However, it is possible to make some predictions of where nuage should be during



Figs. 6–7. (6) A PGC in the genital ridge of a 13-day rat embryo. The nucleus contains little heterochromatin while the cytoplasm contains abundant ribosomes but relatively few profiles of ER. The scale line equals 1 μm. (7) A region of the cytoplasm of an oocyte from a 13-day postnatal golden hamster. Two forms of nuage are present, the solitary, rounded structure (indicated by the double arrows) and the more electron-dense accumulations lying in the interstices of mitochondrial clusters (single arrows). The scale line equals 1 μm. (Fig. 7 reprinted by permission (5)).

embryogenesis if it is a marker for the germ cell line. For example, nuage would have to be present in all of the first 8 blastomeres, since each can apparently give rise to somatic and germ cells. Likewise, some or all of the cells in the ICM of $3\frac{1}{2}$-day embryos, in the epiblast of $4\frac{1}{2}$-day embryos, and in the posterior end of the primitive streak of 7-day embryos should contain nuage because of their apparent dual somatic cell/germ cell potential (see below). Finally, the nuage should be present in the alkaline phosphatase positive PGCs when they are first detected in 8-day embryos.

5. Cell potential and origin of the germ line

The purpose of the germ cell line is to give rise to cells with the capacity to develop into a new individual by the formation of ectoderm, endoderm and mesoderm, and thence the derivative tissues and organs

of these germ layers. At some point during this process, it is necessary to produce the germ cell line in order to assure the next generation. We have seen that in some animals the cells which give rise to the germ cell line are identifiable from the very beginning of development. In these animals, cytoplasmic granules are often present in the cells which are responsible for giving rise to the germ cell line. A variety of studies indicate that these cytoplasmic bodies either contain or serve as markers for the germinal determinant. However, in some other animals such cytoplasmic bodies have not been found consistently throughout the early stages of development. There has not been a demonstration of continuity of transmission of specific components from the egg cytoplasm to precursors of the germ cell line in blastulae and then into PGCs identifiable in gastrula stage embryos. This failure to identify a marker for precursors to the germ cell may be either because the germ cell line is not continuous in these animals, PGCs arising *de novo* by epigenesis (3), or because we have not yet learned how to recognize the germ line markers at all stages. Indeed, it is only quite recently that such germ line markers were found in nematodes (8). Because of this it remains to be seen when the precursors to the germ cell line are formed in such animals as urodeles, chicks and mammals. However, in mammals, recent studies on cell potential in embryos provide information which allow one to predict where such germinal determinants should be located throughout early development, if indeed they are involved in the origin of the germ cell line,

The experimental approach commonly used to determine the developmental potential of specific cells from mouse embryos is to isolate those cells from embryos of a strain carrying particular genetic markers and to test the ability of those cells to form various tissues containing those markers when transplanted into embryos with other genetic markers at those loci. Such cell transplantation studies have indicated which portions of the embryo have the potential to form particular cells and tissues and have suggested where the cells that give rise to the germ cell line are located.

It appears that all of the early blastomeres are totipotent (i.e., can give rise to all components of an embryo). It has been known for some time that each cell from a 2-cell mouse embryo can by itself develop into a mouse (61). More recent studies have shown that the descendants of an individual blastomere isolated from a 4-cell or 8-cell stage mouse embryo and combined with the blastomeres of a host embryo can colonize all tissues in the chimeric mouse that develops (62). Furthermore, these studies have indicated that the germ cell line can arise from any of the blastomeres randomly selected from early cleavage stage mouse embryos, suggesting that the germ cell line precursors are not set aside from somatic cell precursors during this initial period of development. However, it has also been pointed out that these experiments disturb the process of cytoplasmic segregation in the egg that occurs in a regular manner in normal cleavage (63). It remains a formal possibility that experimental manipulation may redirect labile developmental processes that would otherwise result in earlier selection of developmental pathways leading to restriction of cell potential.

The first obvious dichotomy of cell fate in the mouse embryo occurs between the 8-cell and 16-cell stages of development. It has been suggested that microenvironmental differences are responsible for influencing the determination of blastomeres during this period (64) with those on the outside becoming more likely to form trophectoderm cells, which give rise to extraembryonic tissues, and those enclosed by other cells becoming more likely to form the inner cell mass (ICM) which forms the embryo proper. This has been named the 'inside/outside hypothesis' (65). Serial sectioning has demonstrated that inside cells do indeed first appear between the 8-cell and 16-cell stage of development (66), and other studies have shown that the separation of the embryo into trophectoderm and ICM is completed by the 64-cell stage (reviewed in 67). Transplantation experiments using radioactively or genetically labeled cells from cleavage stage embryos have shown that cells placed on the outside tend to become trophectoderm and cells placed on the inside tend to become ICM (66, 68). Although it has not been shown whether determination of trophectoderm and ICM is gradual or abrupt (69), it appears that the trophectoderm cells undergo a specialization or restriction in potential while the ICM cells remain relatively unrestricted. It has been found that single trophectoderm cells from $3\frac{1}{2}$-day embryos (early blastocyst stage) transplanted into host blastocysts have only the potential to form trophectoderm, while ICM cells transplanted under the same conditions were able to produce both embryonic and extraembryonic tissues (70).

The next separation in cell fate during embryogenesis is apparent in the $4\frac{1}{2}$-day mouse embryo (late blastocyst stage), the segregation of the ICM into epiblast and primitive endoderm. Cells transplanted from epiblasts of $4\frac{1}{2}$-day embryos into $3\frac{1}{2}$-day blastocysts have given rise to tissues normally derived from either ICM or trophectoderm. However, primitive endoderm cells transplanted in the same manner give rise only to extraembryonic tissues (71). These