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CONTROL of EMBRYONIC GENE EXPRESSION

M. A. Q. Siddiqui

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Editor

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PREFACE

Recent studies in well-defined systems have disclosed many new facts concerning biochemical and physiological aspects of fertilization, morphogenesis, and development of various forms of life. The crucial area of ignorance in our understanding of embryology, however, is the mechanism that controls these events. Modern embryologists are faced with the task of defining and interpreting the classical embryological concepts on induction, determination, pattern formation, etc. in molecular terms. The goal is to provide a satisfactory basis for understanding the mechanisms whereby an individual cell develops and maintains the diversity of cell types characteristic of the adult state out of the original single cell, the fertilized ovum.

The present state of knowledge strongly supports the view that development and differentiation is a function of variable gene activity. Although we are still on the threshold of knowledge about genes and their functions, it is becoming clear that a coherent view, at least for a few fundamental processes involved in regulation of gene expression, might emerge. It was desirable, therefore, to assemble the available information on molecular biology of early embryonic development and present it in this book as a comprehensive collection of existing data with the pertinent literature review and the evaluation. The book also serves as a useful guide for understanding the basic embryological concepts, which are discussed in a manner intelligible to newcomers to molecular embryology. In addition, it conveys effectively the substance of experimental approaches for future investigations. The subject matter of each chapter naturally reflects the interest of the author engaged in the particular domain of research. The first two chapters discuss the complex regulatory mechanisms underlying fertilization in multicellular organisms. The following chapters attempt to present the fundamental issues concerning the regulation of development. The potential of RNA as a developmental signal is the focus of several articles.

I am greatly indebted to all the authors for making a conscientious effort to provide sufficient background information and present in-depth analyses of the work with a balanced presentation of facts, concepts, and projections. I am also indebted to Ms. Katherine Tighe for secretarial assistance.

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THE EDITOR

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Dr. Siddiqui was elected to memberships of the American Society of Biological Chemists and the International Cell Research Organization. He is also a member of the International Society of Differentiation and Society for Developmental Biology. He has lectured as an invited speaker at many international meetings and symposia and has organized and conducted workshops on molecular cloning at the Institute of Developmental Biology, Peking, China, and at the Institute of Biochemistry, Universidad Austral de Chile, Valdivia.

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Chapter 1

THE ROLE OF THE EGG SURFACE IN DEVELOPMENT

M. R. Dohmen

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I. INTRODUCTION

In a number of recent models the cell surface functions as the main site of regulatory activity in differentiation and pattern formation. Brunner[1] has proposed a model in which the interaction between the cell membrane and the genome explains how differential transcription is directed. He assumes that differentiation is triggered by signals from the microenvironment of the cell rather than by the expression of an endogenous "master" program in the genome. These signals are perceived by receptors in the membrane and relayed to the genome. The genome is programmed to produce a certain sequence of receptors in the plasma membrane. At each stage of differentiation receptors are expressed for the perception of that signal which induces the progression to the next differentiation stage. Membrane-mediated control of differentiation is also assumed in the model of Sumper,[2] which explains certain aspects of the differentiation and pattern formation in *Volvox*.[3] This model is based on the assumption that specific recognition proteins, able to mediate cell-cell contacts, are sequentially expressed at successive cleavages and thus provide a mechanism for counting cell divisions and triggering the differentiations in specific cells. McMahon[4] has proposed a model for position determination in which cells are assumed to interact via contact sensing molecules on their plasma membranes. These respond to cell contact by regulating the intracellular concentrations of a second messenger such as cyclic AMP. The model attempts to explain the development of the pseudoplasmodium of the slime mold *Dictyostelium discoideum* where cells become committed to differentiate into either spore or stalk cells on the basis of their position in the slug.

The emphasis in these models is mostly on cellular interactions and how they bring about differentiation and pattern formation. In this review the focus is mainly on the uncleaved egg, whose surface is thought to be involved in a number of processes that are of vital importance for normal development. These processes include (1) establishing of polarity and regulation of ooplasmic segregation; (2) establishing the conditions for correct fertilization, viz., ensuring species-specific sperm penetration, restricting possible sperm entrance points, and providing blocks to polyspermy; (3) mediating egg activation, the continuation of meiosis, and the onset of cleavage; and (4) determining the cleavage pattern. Of course, not all of these processes occur in every species. In particular, the events associated with fertilization are very variable between different species. The latter events will not be discussed in this review as they are treated elsewhere in this book.

An important aspect of the organization of an egg is its capacity for compartmentalization. It is the basis for differentiation as it provides the initial inhomogeneities that will finally result in cells with different potentials. This mechanism may operate in a direct way by depositing specific determinant factors in certain blastomeres, or it may operate in an indirect way by creating morphogenetic fields or other systems that allow cells to interact in such a way that the proper determinations occur in the right place at the right time. A combination of both processes probably occurs in every species, with the possible exception of mammals and triclad turbellarians, where there are no indications of cytoplasmic localizations in the egg that operate in determination.

Experiments described in the second section of this chapter indicate that the egg surface plays an important role in bringing about or maintaining cytoplasmic inhomogeneities in the egg. This implies that the egg surface itself is not homogeneous and the emphasis in this review is on this aspect.

II. EXPERIMENTS INVOLVING THE EGG CORTEX

A. Centrifugation Experiments

Subjecting eggs to moderate centrifugal forces results in stratification of the cytoplasmic inclusions. Higher forces will separate the eggs in a heavy and a light fragment. It is not

known to what extent endoplasmic cytoskeletal structures or other components that are responsible for endoplasmic organization are resistant to the disruptive centrifugal force. Reports by Conklin[5] on eggs of the mollusc *Crepidula*, and by Lillie[6] on eggs of the annelid *Chaetopterus* indicate that a cytoplasmic "spongioplasm" structure is unaffected by centrifugation. These studies have not been repeated with modern techniques to visualize this spongioplasm, e.g., high voltage electron microscopy or immunofluorescence. It is generally assumed that the egg surface is not displaced by centrifugation, although it may be stretched by the elongation of the egg. The existence of a cortical cytoplasm as a separate entity displaying some rigidity has been doubted in some species, as in centrifuged eggs cytoplasmic inclusions may be found touching the plasma membrane.[7] However, in eggs possessing cortical granules some sort of cortical structure clearly exists, as the cortical granules are not displaced by centrifugation, e.g., in eggs of the sea urchin *Arbacia*,[8] the annelid *Chaetopterus*,[6] and the scaphopod *Dentalium*. A more detailed account of cortical structure will be given in the next section of this chapter.

A large number of reports exists on the effects of centrifugation of eggs on development. It appears that in many species the eggs develop into abnormal organisms after centrifugation, but in many other species the stratification of the cytoplasmic inclusions does not impair normal development. In the latter category, normal development may ensue only under certain conditions. In ascidians, for instance, centrifugation is harmless only when it is carried out on unfertilized eggs.[9,10] In the molluscs *Pholas* and *Spisula* normal veliger larvae develop from eggs in which the first cleavage has not been equalized by centrifugation.[11] In the snail *Lymnaea*, the effect of centrifugation is much more harmful immediately prior to third cleavage than at earlier or later stages.[12]

Abnormal development is generally explained by arguing that the normal position of cytoplasmic determinants in the egg is disturbed by the stratification of the cytoplasm. Ascidian eggs demonstrate this quite convincingly: eggs centrifuged after fertilization give rise to embryos in which all organ primordia are present, but their relative positions are abnormal.[9] Such a clear example can only be provided by organisms showing a highly mosaic type of development, where the determinants for different organ primordia each have a distinct localization in the egg and are independent of each other. In eggs whose development is not purely mosaic, centrifugation may have effects whose causal analysis is far more complicated. To explain the fact that in many species the stratification of the cytoplasm does not preclude normal development, the existence of a cortical scaffold is generally invoked. This scaffold is not supposed to suffer from centrifugation. In Raven's words, the cortex "carries a morphogenetic field, based on local differences in its structure and properties, which has in part a mosaic character. It has been established during oogenesis by the interaction of the oocyte with the surrounding elements of the gonad."[13] If at the time of centrifugation the cytoplasmic determinants are already segregated and rigidly bound to the cortical scaffold, centrifugation will have little effect on further development. This mechanism apparently operates in polar lobe-forming molluscan eggs.

The morphogenetic determinants present in the polar lobe exert a dominant influence on development.[14] This can be demonstrated by removing the polar lobe at first cleavage and studying the resulting development. The larva reared from a lobeless egg invariably shows a large number of characteristic defects. The same defects are observed when the polar lobe of a *Dentalium* egg is removed after centrifugation.[15] This demonstrates that the lobe-specific determinants have not been removed from the polar lobe although the cytoplasm of the lobe may have a composition which is completely different from the normal one. Similarly, in *Ilyanassa* eggs Clement[16] has shown that vegetal egg fragments from which the normal cytoplasm had been removed by centrifugation were able to differentiate lobe-dependent structures. These results clearly demonstrate that morphogenetic determinants may be rigidly fixed to a localized region of the egg surface.

In eggs of the snail *Bithynia* the effects of centrifugation on morphogenetic determinants can be observed directly and the results fully confirm the conclusions drawn from the experiments on *Dentalium* and *Ilyanassa*. In *Bithynia* eggs the polar lobe contains a characteristic aggregate of vesicles called the vegetal body.[17] This aggregate cannot be displaced unless centrifugal forces are employed which disrupt part of the eggs. If these high forces are used, the vegetal body is displaced in about 40% of the eggs. When the eggs are centrifuged before cleavage, they cleave normally afterwards and a polar lobe is normally formed, but this lobe does not contain the vegetal body in a number of eggs. If such a lobe, which lacks the vegetal body, is removed, the egg may develop normally.[14] This suggests strongly that the vegetal body contains the necessary determinants and demonstrates at the same time that there is indeed a cortical mechanism that is able to bind cytoplasmic determinants very strongly.

The difference between the effects of centrifugation before and after fertilization in ascidian eggs can be explained by arguing that localization of determinants in these eggs takes place after fertilization. In fact this can be clearly seen by the appearance of differently colored cytoplasmic regions in ascidian eggs.[18] Centrifuging eggs before fertilization has no effect on morphogenesis as the cortical scaffold exerts its positioning effect afterwards. Once localized, however, the determinants appear not to be as rigidly fixed to the cortex as the polar lobe determinants in molluscan eggs. They can be displaced by centrifugation and consequently disorder results. However, the assumption that there is a rigidly fixed preformed cortical pattern, established during oogenesis and triggered into action sooner or later, is untenable in many cases. A few examples will be discussed. In many species the site of sperm entrance has a definite relationship with the cleavage pattern. It can be argued that both the sperm entrance point and the cleavage pattern are determined by a cortical prepattern. Apart from eggs possessing a micropyle in the surrounding chorions, e.g., in insects, cephalopods, and fish,[19] there are a few reports that the sperm enters the egg at a predetermined site. For example, in *Rana* sperm entrance is limited to the pigmented animal hemisphere[20] and in *Discoglossus* the sperm can only enter through the animal dimple.[21] In ascidian eggs the sperm seems to enter the egg at the vegetal pole,[22] but animal halves can also be fertilized so there is no strict determination in this respect. In the mollusc *Cumingia* and the annelid *Chaetopterus* the first cleavage plane has a tendency to pass through the sperm entrance point. This relationship can be uncoupled by centrifugation. After centrifugation the first cleavage plane tends to parallel the axis of stratification.[23] In the molluscs *Pholas* and *Spisula* the dorsal blastomere D is normally located opposite to the sperm entrance point. After centrifugation the dorsal blastomere can occupy any position respective to the sperm entrance point.[11] In the ctenophore *Pleurobrachia* Freeman[24] suspects that the site where the first cleavage is initiated corresponds to the sperm entrance point. This site indicates the future oral pole of the embryo and the plane of the first cleavage corresponds to the sagittal plane of the embryo. The first cleavage plane can be shifted by centrifugation and the newly established initiation site will be the future oral pole.

Other examples are provided by reversals of animal-vegetative polarity. This axis has been shown in many species to originate from the position of the oocyte in the ovary. The site of attachment of the oocyte generally becomes the vegetal pole of the egg.[25,26] Guerrier[27,28] has shown that in *Parascaris* and *Limax* eggs this initial polarity can be reversed by centrifugation or compression before the extrusion of the second polar body. Before going into a discussion of these results, a survey of other types of experiments will be presented.

B. Micromanipulation and Other Experiments

Probably the best known among these experiments are the graftings of grey crescent cortex in eggs of *Xenopus*.[29,30] Removal of the cortex in the grey crescent region completely prevented morphogenesis, although the removed layer was only 1-μm thick. Grafting the

grey crescent cortex into the ventral side of another fertilized egg induced the formation of a double embryo. In sea urchin eggs more than half of the cytoplasm may be sucked out with a micropipette and still the eggs may give rise to normal plutei.[31] Apparently the endoplasm is relatively unimportant: the cortex contains the vital determinants and this structure is supposed to remain intact in an egg from which cytoplasm is removed. In *Dentalium* eggs 60% of the ooplasm has been removed via a pipette inserted in the vegetal hemisphere of the uncleaved egg. Nevertheless, the eggs developed normally.[32] Similarly, partial removal of the ooplasm from eggs of the ascidian *Ciona* did not impair normal development, though only if carried out on unfertilized eggs.[33] In insect eggs, localized damage to the cortical region or to the blastoderm can produce localized lesions in later stages and this has been used to establish fate maps. The extent to which cortical patterns controlling differentiation can be reestablished following experimental intervention varies; in some species there is considerable flexibility while in the higher diptera and some coleoptera there is little or none.[34] Whether these results should be explained by assuming a mosaic of different and independent determinants or by the action of one or more gradients is still an unresolved question.[35] In *Smittia* eggs, Kalthoff[36] has demonstrated that the anterior determinants are not exclusively localized in the yolk-free cortex. In *Loligo* eggs, regional treatment with small blocks of agar soaked in a solution of cytochalasin B resulted in positionally related effects during organogenesis.[37] In the sea urchin *Paracentrotus* treatment of eggs immediately after fertilization with a detergent resulted in a large percentage of larvae lacking an archenteron, spicules, and mesenchyme cells. These results suggest a cortical influence on the determinants of entomesodermic structures.[38]

All these observations support the view that the cortex is the repository of developmental determinants. However, a number of experiments are difficult to reconcile with the assumption that there is a cortical prepattern. In unfertilized eggs of *Ciona*, partial removal of either cortex or ooplasm has no effect on development.[33] After fertilization, the cortex may still be partially removed without harmful consequences, but removal of ooplasm results in abnormal development. Ortolani[39] has shown that every fragment of the unfertilized or fertilized egg of *Ascidia malaca* and *Phallusia*, if sufficiently large, will give rise to perfectly normal tadpoles, irrespective of the plane of section. Later experiments on *Ascidia malaca* showed that when unfertilized eggs were divided into equal parts by equatorial, meridional, or oblique sections, both parts could be fertilized and developed into complete larvae.[40] In the nemertine *Cerebratulus* unfertilized eggs can be cut equatorially into animal and vegetal halves and then fertilized. Each fragment frequently develops into a normal larva.[41] Thus, the putative cortical pattern may be severely disturbed in some species without impairing normal development.

C. Discussion

It is evident that the egg surface has the capacity to position and bind cytoplasmic components. This is most convincingly demonstrated by the experiments involving the vegetal body of the snail *Bithynia*. The spatial coordinates of the egg of *Bithynia* are predetermined during oogenesis. This can be inferred from the observation that the vegetal body originates during vitellogenesis at the basal pole of the oocyte. This pole is apparently determined to become the future vegetal pole of the egg. There are various other eggs in which the basal pole of the oocyte can be unambiguously identified as the future vegetal pole of the egg.[26] In some eggs this animal-vegetative polarity can be reversed by experimental intervention during a certain period. This means that the polarity, though present, is not rigidly imprinted in the cortex during that period.

The most extensive series of studies on this problem has been done with zygotes of brown algae. In the absence of any other stimulus the apical-basal polarity is determined by the sperm entrance point, but a large array of external stimuli can overrule this initial deter-

mination, e.g., the direction of illumination, electrical currents, centrifugation in combination with pH treatment, and the presence of other eggs.[26] In *Pelvetia* zygotes a local influx of ions and secretion of wall material occur at the presumptive rhizoid site before the polar axis is definitely fixed.[42] If the polar axis is shifted by reversing the orienting light, the local inward current changes accordingly to the new dark side of the zygote within 40 min, followed in about an hour by the deposition of wall material at the new site of inward current. These observations suggest that polar axis fixation results from stabilization of current-producing membrane components. Quatrano et al.[43] have proposed a model in which cytoskeletal filaments act in the transport of membrane components, involved in transporting ions, to the dark side of the light gradient. After having accumulated, they can shift again to another site if the orientation of the light is changed. This process can go on until the membrane components are definitely anchored by cytoskeletal elements.

In animal eggs a similar process may occur. The microenvironment of the oocyte in the ovary serves to initiate a labile apical-basal polarity. This initial polarity can be changed by a variety of stimuli, probably by acting on the cortical cytoskeleton. The timing of the definite fixation of polarity probably differs between species. In *Parascaris* and *Limax* the positioning of the second maturation spindle seems to trigger the definite polar axis fixation. The determination of other spatial coordinates, for example, the plane of bilateral symmetry, is often triggered by the entry of the sperm. A clear example of the initial lability of the spatial pattern induced by the sperm entry is provided by the *Xenopus* egg. The position of the grey crescent is dependent both on the distribution of yolk within the egg and on the point of sperm entry.[44] Its location corresponds to the future blastopore site. Rotation experiments show that the determining effect of the sperm can be overruled.[45] In eggs orientated with the sperm entrance point on top, a blastopore will form in the sperm entrance region, contrary to the rule that the blastopore appears opposite to the sperm entrance point. This reversal occurs even when the experiment is started after formation of the grey crescent as well as shortly before first cleavage. These results imply that the grey crescent may not be the ultimate determinant for the dorsal side and hence Curtis' experiments[29,30] may have to be reevaluated.

The sperm entrance often provokes a visible reaction of the egg surface. Such a reaction may be wave-like propagated from the site of sperm entrance. In *Xenopus* a series of waves has been observed between fertilization and first cleavage. Immediately following fertilization a wave-like propagation of dark-light-dark zones from the site of sperm entrance called the "activation wave"[46] presumably reflects the movement of the front of cortical granule breakdown. Sometime later two "postfertilization waves"[47] proceed from the sperm entrance point to the opposite side of the eggs. In axolotl eggs, just before first cleavage two "surface contraction waves"[48] proceed from the animal pole to the vegetal pole. In *Rana* the pigmented area of the cortex contracts towards the animal pole after fertilization or activation. The function of this contraction might be to move the sperm nucleus towards the animal pole.[20] Nondirectional stimuli may also provoke a cortical response. For instance, a heat shock can induce the appearance of the grey crescent in unfertilized axolotl eggs.[49] These examples demonstrate that the egg surface is a dynamic system that is capable of reacting on stimuli. Such a reaction might unmask a preformed but cryptic cortical pattern, but it is also possible that the stimulus triggers some cortical process that establishes a pattern whose spatial orientation is not preestablished but is dependent on the position of the stimulus, e.g., the sperm entrance point. In view of the experiments described above the latter mechanism seems to be the most plausible one. Still the microsurgical experiments carried out on eggs of *Cerebratulus*[41] and of several ascidians[39,40] remain hard to explain. In these eggs an initial labile polarity is clearly present in the unfertilized egg. In *Cerebratulus* eggs, for instance, a small peduncle frequently marks the vegetal pole of the egg. Fragments of these eggs are each capable of establishing the necessary set of spatial coordinates. A simple shifting of

provisionally localized polar determinants to another site is impossible, because if they were initially localized, they must be absent in one of the two egg fragments. Possibly the initial pattern is not a mosaic of distinct factors. It may be formed by a gradient in which each segment has regulative capacity. Freeman's[24] statement that "eggs from some groups of animals have essentially no promorphological organization at the end of oogenesis" is probably true as far as the localization of cytoplasmic morphogenetic determinants is concerned. However, the eggs are clearly biased to develop along spatial coordinates that are evident already in the unfertilized egg. This implies the existence of a promorphological organization at the end of oogenesis, however labile this organization may be.

III. THE STRUCTURE OF THE CELL SURFACE

A. The Cortical Cytoplasm

In all cells, eggs as well as differentiated cells, a distinct cortical cytoplasm probably exists. In murine fibroblasts[50] the cytocortex is characterized by a high concentration of ground substance and a complete exclusion of all cellular organelles. It contains very short, interconnected threads and electrondense globules, forming a network. In erythrocytes a membrane cytoskeleton, composed of a specific protein (spectrin), actin, and other elements has been demonstrated.[51] Moore et al.[52] have proposed a model in which α-actinin associates directly with the membrane, possibly through interaction with an integral membrane protein. Short actin filaments interact with the α-actinin at one end and are tied together to form a submembrane matrix by cross bridges of actin-binding protein and myosin. Actin-binding protein is thus able to provide stability for the submembrane matrix while the myosin-actin interaction exerts a contractile force that can be used to power membrane movements.

Detailed knowledge of cortical structure has been provided by the study of ciliated protozoa. These organisms have a highly complex and easily observable cell surface organization that might serve as a model system for other organisms in which cortical patterns are often hard to detect. In *Tetrahymena* a distinct cortical layer, the epiplasm, is characterized by two major proteins, probably in association with actin.[53] One of the most spectacular properties of the ciliate cortex is its potential for pattern maintenance. A reversed ciliary pattern, caused by grafting a small piece of cortex into a host after 180° rotation, has been preserved through 1500 fissions in *Tetrahymena*.[54] The mechanism responsible for this cortical inheritance has been termed "cytotaxis" by Sonneborn[55] and "structural guidance" by Frankel.[56] The local cortical environment created by the position and orientation of preexisting ciliary units within a ciliary row determines the position and orientation of new units within the same row. The old structures serve as scaffolds for the new ones.[57] Apart from this mechanism for short-range positioning, there is also a mechanism for long-range positioning in ciliates. The latter mechanism operates through gradients of positional value. These gradients should probably not be thought of as gradients of a diffusible morphogen, as this concept is difficult to apply to a unicellular system. What else we should think of is not clear at present.

The existence of a distinct cortical cytoplasm in eggs was postulated from the observation that in sea urchin eggs the peripheral region shows birefingence.[58,59] A variety of biophysical and micromanipulation studies of the rigidity of the surface have confirmed the existence of a distinct cortical layer in sea urchin eggs. Its thickness has been measured by various methods and the values obtained range between 2 and 6 μm. In a recent study of sea urchin eggs Higashi[60] found that the cortical layer in the fertilized egg is not a gel but consists of a hard matrix. It exists also in the unfertilized egg, but in a more fluid form. Schatten and Mazia[61] have shown by scanning electron microscopy that the cortex consists of an overlapping network of fibers measuring between 50 and 200 nm. The thickness of the cortex, as measured on SEM micrographs, ranges between 0.2 and 0.5 μm, which is far less than the values obtained with other methods. The cortex contains myosin and actin and seems

to cause a spreading surface deformation at fertilization which may be involved in the secretion of the cortical granules, in the detachment of the vitelline sheet from the egg surface, and perhaps in the rapid block to polyspermy.[61] A cortical protein with spectrin-like properties has been isolated from sea urchin cortices by Kane.[62] Harris[63] has observed a spiral cortical fiber system, visible with phase-optics, in *Strongylocentrotus* eggs between 30 and 70 min after fertilization. Franke et al.[64] have studied the cortex of various amphibian oocytes. It appears to consist of a mechanically stable meshwork of microfilament webs, dense aggregates of coiled filaments, paracrystalline arrays of microfilaments, microtubules, and vesicles. Actin is located in the microvilli and in small aggregates in the cortex. The authors suggest that the oocyte cortex contains a considerable amount of actin and microfilaments in storage forms. Long bundles of parallel microfilaments as they have been observed in the cleavage furrows of axolotl eggs[65] are only rarely found in the oocyte cortex.

The mechanism that brings about unequal cleavage is particularly intriguing. Extreme examples are the meiotic divisions which give rise to the polar bodies. The mechanism responsible for the formation of polar bodies does not reside in any particular characteristic of the meiotic apparatus. If a meiotic apparatus is displaced by centrifugation, it may induce the formation of normally sized blastomeres, the so-called giant polar bodies.[5] The meiotic apparatus becomes rigidly anchored to the cortex during metaphase and then the peripheral aster disassembles prior to the internal aster, thus allowing the spindle to move closer to the egg surface.[75] Czihak[76] has suggested that a similar phenomenon possibly occurs during the formation of the micromeres in sea urchin eggs. The vegetal cortex might differentially depolymerize microtubules so that the peripheral asters become smaller and the spindle moves closer to the vegetal pole. In ciliated protozoa the role of the cell surface in positioning the nucleus and determining its behavior has been thoroughly investigated.[77] A particularly striking example is provided by the macronucleus of *Stentor*.[78] This nucleus is located beneath and parallel to the narrowest cortical striping of the cell's right side. When the direction of the striping is changed by microsurgery, the position of the macronucleus is shifted accordingly. In stentors with reversed asymmetry of the cortical pattern, the position of the macronucleus is also reversed. It is concluded, therefore, that the cortical striping determines the orientation of the macronucleus. Franke et al.[64] have shown that there is no prepattern of contractile rings of filaments in amphibian oocytes. The formation of these structures is induced immediately before actual cleavage occurs. The nature of the stimulus is obscure.[79] Calcium appears to play an important role in activating the cortical contractile system, as microinjection of calcium or treatment with ionophores can provoke the appearance of various kinds of cortical contractions.[80-83] The waves of cortical granule breakdown also depend on the presence of calcium. Microinjection of calcium into amphibian oocytes induces dehiscence of cortical granules.[84] In eggs of the medaka a wave of release of free calcium can be shown to precede the wave of cortical granule breakdown.[85]

Apart from the phenomena already described above, there are many other contractile events to be observed in eggs. A prominent example is provided by the constriction of polar lobes in eggs of a number of molluscs and annelids. A contractile ring composed of cytochalasin B-sensitive filaments has been shown to cause this constriction[86] which is independent of any nuclear activity as it occurs also in enucleated vegetal halves.[87,88] In many eggs the maturation divisions are accompanied by extensive deformations. The most conspicuous deformations have been observed in eggs of the annelid *Tubifex*. Shimizu[89] has described two successive deformations during maturation: grooves caused by contracting microfilaments appear first on the equatorial surface, then on the animal hemisphere. In eggs of the barnacle, *Pollicipes polymerus*, a series of constriction rings moves slowly from the animal to the vegetal pole.[90] The constrictions are inhibited by cytochalasin B, but not by colchicine. Pulsations have been described in the eggs of *Nereis*.[91] The pulsations start immediately before the breakdown of the germinal vesicle, about 15 min after insemination,

and proceed continuously with the exception of a short rest period of 3 to 5 min which occurs at about 25 min after insemination. In eggs of the bivalve molluscs *Barnea candida* Pasteels[92] has observed that occasionally undulating movements of the vitelline membrane occur. They may be provoked with greater intensity by cold treatment. These undulations result from successive waves of elongation of the microvilli. This elongation does not involve any undulation of the plasma membrane. Rhythmic movements of the fertilization membrane have also been observed in the eggs of *Chaetopterus, Mactra,* and *Nereis.*[93] The function of this surface activity is generally obscure. A role in cytoplasmic localization or in egg activation seems to be plausible. However, when the peristaltic constrictions in *Pollicipes* eggs are inhibited by cytochalasin B, ooplasmic segregation continues, and when ooplasmic segregation is inhibited by antimycin A, the constriction continues.[90]

B. The Plasma Membrane

The prevailing model of plasma membrane structure[94] depicts the membrane as a fluid lipid matrix in which integral proteins are embedded, whereas peripheral proteins are bound weakly to the surface. The lipid matrix is responsible for the basic membrane structure and the proteins are considered to be responsible for specific functions. A single phospholipid such as phosphatidylcholine could satisfy the structural requirements of the basic structure.[95] The observation that the membrane of a single cell may contain as many as one hundred or more different lipids suggests that lipids play other functional roles. The ability of lipids to adopt nonbilayer configurations in addition to the bilayer phase provides a variety of possibilities for the direct involvement of lipids in many functional abilities of biological membranes.[95] However, as far as specific functions are concerned, most data refer to membrane proteins. In accordance with the fluid nature of the membrane, most membrane proteins are randomly distributed over the cell surface, as far as can be judged from experiments in which the exposed proteins or carbohydrate groups were labeled with antibodies, lectins, anionic or cationic probes, etc.[96] The mobility and distribution of membrane proteins are thought to be affected by cortical microfilaments and microtubules, and this may result in nonrandom patterns. Well-known examples of stable patterns of membrane components are found in the intercellular junctions. The different types of junctions are characterized in freeze-fracture preparations by organized arrays of inner-membrane particles (IMP).[97] Other examples are found in the ciliated protozoa *Tetrahymena* and *Paramecium* which display two types of stable repetitive IMP arrays, viz., ciliary "necklaces" and trichocyst or mycocyst insertion sites, regularly alternating over the cell surface.[98] Examples of nonrandom distribution of antigens, surface immunoglobulins, and anionic sites have also been reported.[96,99]

Redistribution of membrane components can be brought about in many ways: by temperature changes, alterations in membrane lipid composition, pH changes, enzymes, membrane-active drugs, labeling of surface antigens, lectin receptors and anionic sites with appropriate ligands, etc.[96] The involvement of cytoskeletal elements is suggested by several observations of striking correlations between patterns of membrane components and patterns of underlying cytoskeletal elements. For example, in fibroblasts the concanavalin A receptors can be induced to form linear arrays of clusters which appear to be aligned with intracellular stress fibers.[100] Singer[101] observed a correlation between linear or fusiform aggregates of pinocytotic vesicles on fibroblasts and cortical microfilament bundles. Elgsaeter et al.[102] found that the aggregation of spectrin in erythrocyte ghosts correlated with aggregation of IMP. Even peripheral proteins such as fibronectin, which is loosely associated with the external membrane surface,[103] seem to maintain transmembrane connections with the cortical cytoskeleton. Double-label immunofluorescence has shown a definite correspondence between the fibrillar arrays of fibronectin and intracellular actin.[104] Analogous investigations have failed to detect any relationship between fibronectin and microtubules or intermediate filaments. As neither actin nor fibronectin are integral membrane proteins, there must be intervening proteins connecting the two.

The role of cytoskeletal elements can be experimentally demonstrated by disrupting them with colchicin, cytochalasin B, local anesthetics,[105] or other agents. After disruption ligand-induced redistribution of surface receptors is facilitated.[95,105] Disassembly of microfilaments by cytochalasin B results in release of fibronectin from the cell surface.[104] The current view is that microtubules and microfilaments play opposing roles in the distribution of membrane components. Microtubules, in this concept, serve to anchor the components and microfilaments tend to redistribute them by their contractile activity. The interplay between these two opposing but coordinated systems determines the topography of the membrane.[96,99]

Eggs are highly specialized cells and we may expect that this is also manifested in the properties of the plasma membrane and in its modulations during development. One of the properties that can be investigated is the lateral mobility of membrane components. This can be accurately measured by means of the fluorescence photobleaching recovery (FPR or FRAP) technique.[106-108] This technique involves labeling a specific cell surface receptor with a fluorescent ligand followed by laser-induced bleaching of the fluorescence in a small region. The time required for the recovery of the original amount of fluorescence in this region is a measure of the rate of lateral diffusion of neighboring unbleached fluorophores into the bleached region. In mouse eggs Johnson and Edidin[109] measured the diffusion of antigens in this way and found a very high diffusion coefficient in the unfertilized egg. After fertilization a dramatic reduction was observed. On the other hand, in sea urchin eggs an increase in membrane fluidity was observed after fertilization.[110] The latter measurements were done by using a spin label fatty acid, so the fluidity of the lipid matrix is measured. In FPR measurements of the lateral mobility of proteins the fluidity of the lipid matrix may be of minor importance if the mobility is restricted by cytoskeletal elements. The results obtained by Johnson and Edidin[109] may thus reflect a sudden restriction of lateral mobility by anchoring of the antigens to cortical elements following fertilization, independent of any change in lipid matrix fluidity.

Evidence for a high degree of stability of membrane components is provided by Bluemink et al.[111] who showed that in *Xenopus* eggs the preexisting and the nascent membrane are continuous but nevertheless maintain their highly different IMP densities. Bluemink and Tertoolen[112] suggest that IMP-associated filaments probably are instrumental in creating long-range stability in the plane of the membrane. These filaments may control cell surface topography and as such they may function in establishing polarity and bilateral symmetry in the amphibian egg. Other examples of stable patterns have been found in sea urchin eggs. After fertilization the membrane of the cortical granules is incorporated in the plasma membrane. The mosaic membrane thus produced is composed of smooth patches, derived from the cortical granule membranes, and microvillous patches, derived from the original plasma membrane. This mosaic surface is then reorganized by the elongation of microvilli and by the reduction in size of the smooth patches. The mosaic character of the surface is still recognizable after microvillar elongation, indicating that the different membranous components do not intermix.[113] However, recent measurements of surface area indicate that the mosaic membrane is not a long-lasting composite structure. A large area of egg surface is resorbed within 10 min after fertilization.[114] A considerable degree of stability in sea urchin and mouse eggs is demonstrated by the persistence of the sperm plasma membrane as a discrete patch in the egg membrane.[115] This shows that there is a limited lateral mobility and a low turnover of surface components in these eggs.

If the spatial organization of the egg is primarily under the control of the egg surface, one might expect to find patterns of membrane components that correlate with animal-vegetative polarity and cytoplasmic localizations. These patterns have only rarely been found. In *Xenopus* eggs the overall densities of IMP in the animal and the vegetal hemisphere do not differ significantly.[116] However, for IMP sizes smaller than 81 Å, a significant difference was found, more small IMPs being present in the animal hemisphere. Labeling of externally