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TIME, SPACE, AND PATTERN IN EMBRYONIC DEVELOPMENT

Editors
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Preface

Embryology appears to be unique among modern experimental disciplines in biology in not only still possessing but still celebrating its ancient unsolved problems. One of the most important as well as one of the oldest of these is the origin of complex morphology from an apparently formless egg. The first mechanistic hypotheses directed toward the solution of this problem were framed in the 18th century and suggested that the egg contained a miniature, preformed "homunculus". Development was thus seen as the unfolding and growth of an already existing structure, and the miracle of epigenesis, or form arising spontaneously, was avoided. Unfortunately, this idea could not be maintained in the face of the careful embryological investigations of the late 18th and early 19th centuries. There was no homunculus, and epigenesis appeared to be inescapable.

As has been pointed out by Ernst Mayr in **The Growth of Biological Thought**, strikingly opposed viewpoints in biology are often ultimately resolved in a synthesis. In the case of preformation vs. epigenesis, structure does appear de novo, but it is also true that preformed directions for the generation of structures are present in the egg. No one today, of course, has any difficulty recognizing those instructions as those of the DNA genome present in the egg pronucleus. There is, however, a source of preformed information in eggs distinct from and complementary to genomic DNA. It was recognized in the late 19th and early 20th centuries from studies conducted at the Marine Biological Laboratory (MBL) in Woods Hole and elsewhere that the cytoplasm of eggs and early cleavage stage embryos was not isotropic. Instead, it was found that informational molecules or determinants, localized in specific regions of the cytoplasm, were crucial for correct differentiation of the blastomeres containing them.

The relationship of localized cytoplasmic determinants to the action of the nuclear genome in development posed a conundrum. For example, F.R. Lillie, in a lecture delivered at MBL in the summer of 1927, envisioned embryonic development to be entirely directed by selectively localized cytoplasmic information because all cells contain the same genome. Our current view that localized determinants act by influencing nuclear gene expression in particular regions of the embryo was first explicitly stated by T.H. Morgan in 1934 in his book **Embryology and Genetics.**

The chapters in this book are derived from lectures which constituted the central theme of the Embryology course at the MBL in the summer of 1982.

The book presents the current state of investigation on cytoplasmically localized information in embryos, its spatial distribution, possible molecular nature, time of expression, and relationship to the function of genes involved in pattern formation. Although many of the topics considered here have long histories, it is apparent that recent investigations are at last beginning to produce significant new insights.

Localization phenomena are dynamic processes linked to the cleavage cycle and to other temporal control systems in the embryo. Investigations with the embryos of spiralians, nematodes, insects, and in nonmosaic mammalian embryos reveal a considerable evolutionary flexibility in the management of determinative processes during early development. In some instances, such as the germ-line determinants of insects, determinants are already positioned in the region of the egg in which they will ultimately function during oogenesis. Regionalized information systems already established in the egg may determine the cleavage patterns during early development. However, in most embryos, determinants reach their final positions via a set of progressive cytoplasmic movements as cleavage proceeds. In mammalian embryos, preformed determinants appear not to exist or at least to play no demonstrably significant roles in early decisions. Instead, interactions between cells and relative location of cells in the cleaving embryo provide the information for determinative decisions.

The chemical identities of localized determinants are still poorly understood. However, recent advances in nucleic acid technology, notably the use of cloned DNA sequences to probe for specific mRNAs in fractionated embryos or in sectioned embryos in situ, have made it possible to map the spatial and temporal distributions of particular mRNAs. The use of monoclonal antibodies makes it possible to launch a similar search for potential protein determinants. Some rather surprising results have emerged from these studies. Both maternal mRNAs and proteins exhibit discrete spatial localization patterns. Perhaps of equal importance is that the localization and translational expression of mRNAs are under temporal controls. The finding that molecular events such as the release of physically sequestered maternal mRNAs is controlled by timing mechanisms is particularly exciting because there is ample evidence that the functioning of developmental determinants is tied to the cleavage clock. For example, the timing of micromere formation in sea urchin embryos is specified by the cleavage clock whereas the site of micromere formation is specified by information localized in the vegetal portion of the egg. In some cases, the cleavage clock can be uncoupled from cleavage. Thus, if cleavage is delayed by one cycle relative to the clock, sea urchin embryos can be made to produce micromeres at the correct clock time but at the third instead of the fourth cleavage.

A final conceptual and experimental advance stems from the developmental-genetic analysis of pattern formation in *Drosophila*. The initial pattern (i.e., anterior-posterior, dorsal-ventral) is determined by genes active during oogenesis. Such maternally established patterns are interpreted and elabo-

rated by genes active in the embryo. These include genes whose action is spatially regulated by maternal patterning elements to establish the number, location, and polarity of segments. Genes revealed by homoeotic mutations establish segment identities. Cloning of these genes is beginning to provide a molecular approach to the action of genes so far accessible only by genetic and developmental analysis.

The classic problem of the origin of form in developing embryos remains, but efforts such as those presented in this volume not only have revealed much about the temporal and spatial functions of information systems present in the early embryo but also have allowed the problem to be cast in new, experimentally accessible terms. The problem we face (in evolution as well as in embryology) is one of accounting for how the activity of genes is converted into morphology. Development presents us with a sort of black box in which the crucial processes occur. Clearly there is more to it than the relatively well-understood processes of transcription and translation or the self-assembly of proteins into higher-order structures. The genes that act during oogenesis to establish pattern dictate the subsequent spatial and temporal events related to gene expression. Current methodologies are begining to allow us to document and study such events. The relationship between localized or temporally specified mRNAs and morphogenesis is still obscure, but the existence and involvement of a complex cytoskeletal architecture in mRNA localization in eggs and embryos suggest a crucial role for cell structure in the expression of genes in development. The complementary use of genetic, molecular, and cell biological techniques to attack the problems posed here is illustrated by the chapters in this volume. We hope that this book proves as exciting to readers as it has been to us. Perhaps the best mark of the book's success will be if it helps to stimulate research which will quickly render it obsolete.

No project of this kind grows in isolation. We have profited from the encouragement and help of many of our colleagues and students. In particular, we wish to thank Paul Gross for providing the resources of the MBL which made possible the rich lecture series that underlies this book. We also thank Joan Howard and many others of the MBL staff for their resourcefulness and cheerful help in making the Embryology course work smoothly, and Alan R. Liss and Paulette Cohen, who provided some of the initial suggestions for this project. Finally, we are grateful to our students and colleagues in the Embryology course for the intellectual stimulation and good companionship they provided throughout the summer, and to our coauthors in this volume for their superb lecture presentations and for their cooperation in writing and editing these chapters.

Rudolf A. Raff
William R. Jeffery

Foreword

Among the research interests of the founders of the Marine Biological Laboratory in 1888, embryology was second to comparative morphology and physiology. Yet within a very short time—five years, more or less—certain issues of embryology came to dominate discussion and investigation during the intense and productive summer sessions of the Laboratory. This was in no small measure a consequence of the personalities and intellectual strengths of C.O. Whitman, the first Director of the Laboratory, and E.B. Wilson, followed by such younger colleagues and students as E.G. Conklin, T.H. Morgan, and F.R. Lillie, who was to succeed Whitman, after the latter's brilliant tenure as Director of the MBL.

A formal course in embryology was established in 1893 in response to considerable pressure from many quarters for such an effort. Its main virtue would be to bring together in one place, for the benefit of serious students and younger investigators, a faculty of quality not duplicable in any single university, with an abundance of biological material uniquely suited to the studies of interest. The establishment of the Embryology course followed by five years the founding of the Laboratory and of its first course offering, Invertebrate Zoology. (Marine Botany was first given in 1890, and General Physiology—the first such course in the world—in 1892.)

The Embryology course, whose 1982 lectures are the chapters of this volume, has thus been offered essentially without hiatus since its founding. Its 89-year history is surely as long and distinguished a record as that of any science-teaching activity in the world. Those who are acquainted with scientific pedagogy, however, as well as with the history of science, will find in this some contradiction.

Most of us are aware that a science course offered unchanged even over half a decade is likely to be obsolete in most of its parts; lecture notes a decade old, however nicely retyped, are useful for little more than nostalgia. How, then, can the MBL's Embryology course have survived, in its primary organization (as it has), for the better part of a century, and yet have been continually "distinguished"?

The answer is that it has always been a kind of sculptor's armature, cleaned of dry, adherent clay every winter, and remodeled by the faculty and the leading thinkers of the field every summer, according to the highest

current canon. That is, indeed, true of all the MBL summer courses, so that their titles are sometimes a less-than-explicit guide to their contents. Aside from this little problem of titles, the system has worked well. Its product has been an educational program that is evolutionary in the best sense: a scientific training in which there is continuity with change, the useful changes stabilized by selection pressure. Ideas, techniques, arguments that do not work well, or prove to be of low relevance to the central problems, fail to reproduce themselves. Important ideas and methods, issues that demand resolution independently of immediate changes of style or technique, reproduce themselves and are themselves subject to further evolutionary change.

The issues toward which the 1982 lectures were directed, and these chapters based upon them, are a splendid example of the process. At the base of all is a transcendent issue and problem, one of the first such problems to be identified with the MBL and with American biology quite specifically: the physical basis of determination in early animal development.

Out of it came some of the finest biological research of the late 19th and early 20th centuries, much of it done or inspired by the founders of the MBL. They did not solve the problem, but they did reformulate it eventually in such terms as to make it honestly investigable. That was a powerful advance over the natural-philosophical notions of the driving forces of embryogenesis that had become dominant in Europe during the preceding thirty years.

Yet the chapters of this volume deal with methods and fundamental concepts that would have been unimaginable to the MBL's founders—methods and concepts that are in fact remote from those that dominated "experimental embryology" and "developmental genetics" as recently as the 1940s and 1950s. I can imagine no more poignant lesson in the excitement, the aesthetic excellence of good biological research, and at the same time its awful transience, than to read these chapters side-by-side with lectures, papers, and books produced by MBL embryologists just a few decades ago. The work must indeed be its own reward: that, too, we try to teach students at the MBL.

The first line of entirely original, internationally influential research at the new seaside laboratory in Woods Hole was a kind of investigation dubbed "cell lineage." Exploiting energetically the lead provided by Van Beneden and others in Europe during the 1870s and 1880s, Director Whitman, in powerful work begun as his doctoral dissertation of 1878, established the existence of a precocious bilateral symmetry and of organ-specific regions in the egg of the leech *Clepsine*. E.B. Wilson did likewise for the clamworm *Nereis*, and that was followed, among others (but none so elegantly), by the beautiful studies of Conklin on predetermination as manifested in cell-lineages of the *Crepidula* embryo.

These investigations and their offspring appeared unequivocally to refute antecedent notions of phylogeny and germ-layer homology as the *specific*

processes driving early embryogenesis; and, more importantly, to disprove the proposal of Weismann that early nuclear (i.e., genetic) differentiation—a direct consequence of cleavage— is the agency of cytoplasmic divergence among the early blastomeres. The groundwork was provided for what was to become a transcendent idea: that the cytoplasm of an egg, or of a zygote, is regionally inhomogeneous, and that it is to that extent possessed of information not implicit simply in the (average) chemistry of the "protoplasm."

Nor was the experimental evidence limited to the "mosaic" eggs of molluscs, worms, and tunicates: it was not long before such simple but powerful experiments as those of Morgan on centrifuged sea urchin eggs demonstrated that even "regulative" embryos begin development with an axis of polarity, at least, preformed.

There were then, as there are today, tensions among groups of investigators of different disciplinary background. The rising and brilliant group with allegiance to the General Physiology course and to the leaders of that field, most notably Jacques Loeb, found (not entirely without justice) some tendency toward vitalism, or at least a smell of it, in talk of "organ forming substances," or later, of "morphogenetic determinants"; in the idea that the "protoplasmic organization" contains a blueprint, or program, for the three-dimensional reality that is the larva of an invertebrate animal.

On the other hand, the embryologists, closer to the biological material and with an unshakeable sense of the spontaneity and *directedness* of cleavage and morphogenetic movements, saw in the notion of "isotropy" of the egg, as put forward originally by Loeb and others, an example of simplistic, premature emphasis upon chemistry and physics, to whose primacy the physiologists were committed on philosophical grounds.

Driesch's success in separating the early blastomeres of sea urchin embryos was a great boost for the argument of "isotropy." It seemed to refute the possibility that *any* predetermination of morphologic outcome can exist, at least up to the four-cell stage. (At the same time, Driesch's result was a further blow to Weismann's proposal of nuclear differentiation and divergence.) It remained for Wilson and Morgan, and for two entire generations of their followers at the MBL and elsewhere, to show that there are *not* two perfectly separable and distinct kinds of development, "mosaic" and "regulative"; that the sea urchin egg is *not* isotropic; and that the molluscan and annelid eggs are *not* perfectly and finally predetermined to the last feature of larval form and function.

Simultaneous with the rising conviction that to determine when and how *genes* function in development (in a discipline that came to be known as "developmental genetics") is an indispensable step in the analysis of embryogenesis, there was established the certainty that all or nearly all animal embryos have their cleavages guided and their emerging form determined by cytoplasmic elements, independent of the nuclear genes. It was a convincing

argument of E.B. Wilson's monumental textbook (third edition in 1925) that while the "morphogenetic determinants" may not be in their final locations at the very start of development, and may indeed be redistributed in various ways after cleavage is underway, most if not all early development is directed by them, in the sense that the fundamental symmetries and the developmental potencies of blastomeres result from their presence or absence in a particular place.

The contemporary era (which I suspect is nearly at an end, to be followed by an even more extraordinary one of *directed* transformation of embryos) began with the mutual interaction of molecular genetics—itself an offspring of formal genetics and microbial physiology—and experimental embryology. One can set the time as the middle 1960s. There was no longer any question about whether or not genes have something to do with development, and if they do, whether or not it is early. It was by that date clear that genes have *everything* to do with development, in the broadest sense; because, among other reasons, at every step of the way the last step of gene expression—protein synthesis—must occur. Inevitably, in the new interface between molecular genetics and embryology, the old issue, settled in principle but certainly not in chemistry, came once again to the fore.

What is a "morphogenetic determinant"—in chemical terms? Where is it located? How does it work? How is its information content related to the program explicit in the nuclear genome? What relationship exists, if any, between it and the emergence of *form*—between it, in short, and the spontaneity and directedness that so astonished the first embryologists who followed cell lineages; and that so enchant today's high-school student, set down before a microscope with a clock, a culture of newly fertilized marine invertebrate eggs, and, perhaps, a sandwich and a book?

This is an old issue, as indicated, but it is one to which every trick of four trades—descriptive embryology, biochemistry, molecular genetics, molecular cell biology—has been applied with profit. That, in fact, is what this book of lectures is all about. I leave it to the reader to judge the quality of the work and the amount of progress made, should he or she be familiar with the way things were just a few years ago. I say only that there *are* morphogenetic determinants in the cytoplasms of early embryos, of nearly all kinds. The only likely exceptions are those of birds and, as implied in the Preface to this volume, mammals. I accept even that qualification with suspicion: it seems to me to be stretching homology to equate all the early blastomeres of a mammalian zygote (as opposed to the first cells in the inner cell mass), or the products of incomplete cleavage in the avian blastoderm, with early blastomeres of an annelid, or echinoderm, or a tunicate.

There is, moreover, an enormous amount of information stored in the cytoplasm of an egg, over and above that to be found generally in the cytoplasm of a somatic cell or a late embryonic cell. Some of it is in the form of RNA transcribed during oogenesis, and some of *that* is messenger RNA, responsible for directing a large part of the protein synthesis during early development. Some of the RNA is apparently *not* mature messenger RNA, but is rather a complex set of macromolecules of still unknown function, containing interspersed repetitive sequences found otherwise only in the nuclear RNA of somatic cells. As Eric Davidson and his colleagues have shown, these transcripts of genes are not likely to be accidentally present in the egg; they are a specific subset of the nuclear sequences, and probably have a specific information function.

There are also cytoplasmic proteins, some of them apparently released from the germinal vesicle at the time of its breakdown, that direct or facilitate morphogenetic processes in later development. And there appear to be, remarkably, architecturally-differentiated domains of the egg cytoplasm, as evidenced by differential organization of the molecular cytoskeleton, that match the domains of those morphogenetic "plasms" so painstakingly traced out by the first investigators of cell lineage.

From the point of view of molecular biology, in fact, the uncleaved egg contains an information-rich, asymmetrically-distributed *secondary genome*; relatively long-lived by the time-scale of early development; some of whose ultimate products (e.g., chromosomal proteins) almost certainly influence subsequent gene expression in the cells to which they are sequestered by cleavage.

In a sense, if and when these last assertions are known—and generally accepted—to be true, the long quest upon which the MBL's first Director embarked will have been completed (by his descendants, of course). But as is always the case in basic science, the end is just a beginning. Of what it is a beginning will undoubtedly be a major interest of next summer's Embryology course at the MBL; but it is also announced, explicitly or by implication, in the inspiring chapters that follow.

Paul R. Gross
Woods Hole, Massachusetts
April 1983

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Fusion of Sea Urchin Eggs

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Little is known about the contributions of different parts of the egg to the more advanced embryo or adult organism. This is in part due to the fact that few species exist in whose eggs there are natural markers which can be followed over the course of development. From studies following the fates of those egg markers that do exist (such as the yellow pigment in certain ascidian eggs [Morgan, 1927], the red pigment band in eggs of one species of sea urchin, *Paracentrotus lividus* [Morgan, 1927], and the gray crescent in the frog egg [Ancel and Vintemberger, 1948]), we know that defined regions of the egg cytoplasm can give rise to particular structures during the course of embryogenesis. However, we still know very little about the interactions between cytoplasmic domains *within* the eggs themselves. In order to investigate what types of information might be transmitted or shared between different realms of the egg, we devised a method of fusing eggs together. We chose to work with two types of eggs which have numerous gross morphological (and biochemical) differences: fertilized and unfertilized sea urchin eggs. By using donor eggs from sea urchins of different species *(Strongylocentrotus purpuratus* and *Lytechinus pictus),* we can recognize the two cytoplasms by light microscopy. With this system, we attempt to examine the structural integrity of the cytoplasmic, surface, and nuclear components of the egg.

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FUSION OF SEA URCHIN EGGS

Descriptions of fused sea urchin eggs appearing spontaneously in a normal population of unfused eggs exist in the literature. Fused eggs have been observed either at the end of the spawning season or after treatments designed to remove the fertilization envelope [Driesch, 1900; Bennett, personal observations]. Such spontaneous fusions are very infrequent and it is not possible to predict when or whether they will occur.

The experimental induction of the fusion of sea urchin eggs has also been reported. Several different methods have been used. These are: (1) to treat the eggs with Ca-free seawater after removal of the fertilization membrane [Driesch, 1900], (2) to treat eggs with alkaline seawater and then to centrifuge them [Driesch, 1900; Bierens de Haan, 1913a,b], (3) to place eggs in hypotonic solutions of NaCl in seawater [Goldfarb, 1913], (4) to centrifuge eggs together in capillary tubes [Tyler, 1935, 1942], and (5) to apply an inhomogeneous alternating electric field to eggs in a nonconductive medium [Richter et al., 1982]. Although a few of the eggs treated with these various methods were described as being fused, only in the last situation were the two separate plasma membranes really united rather than just tightly apposed.

Recently we developed a new technique which furnishes fair numbers of fused eggs and permits the fusions of unfertilized and fertilized eggs of different species [Bennett and Mazia, 1981a,b]. This technique takes advantage of the ability of positively charged polymers to induce adhesion of the negatively charged eggs to one another. When then immersed in a medium conducive to fusion, many of the adherent eggs merge [Bennett and Mazia, 1981a]. In this chapter, we will demonstrate what happens when two cells with very different and very well-studied morphological and biochemical characteristics (i.e., fertilized and unfertilized sea urchin eggs) are fused. We will discuss the questions: (1) Is the autonomy of one egg maintained when it is fused to another? (2) Is the fused egg intermediate in its characteristics or does it have characteristics of just one or the other original eggs? (3) Is the unfertilized portion fertilized upon fusion? and (4) What happens at mitosis?

SEA URCHIN EGGS—BACKGROUND

At the time of spawning, unfertilized sea urchin eggs have completed metaphase II of meiosis. They are in a relatively quiescent G_0 state, awaiting the arrival of the sperm or some parthenogenic agent for entry into the cell cycle [Giudice, 1973; Czihak, 1975]. Immediately upon fertilization or parthenogenic activation, the eggs enter the cell cycle.