The Beginnings of Embryonic Development

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A symposium organized by the Section on Zoological Sciences of the American Association for the Advancement of Science, co-sponsored by the American Society of Zoologists and the Association of Southeastern Biologists, and presented at the Atlanta Meeting, December 27, 1955

Edited by

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PREFACE

A symposium on "Formation and Early Development of the Embryo," held December 27, 1955, at the Second Atlanta Meeting of the AAAS, served as the basis for the present volume. The symposium emphasized problems of early development and of the initiation of development. However, in selecting this general area for discussion the organizing committee did not intend to imply that the problems are considered to be fundamentally different from those encountered in later development. In fact, many of the contributions presented here indicate the generality of the problems of developmental change at any stage along with the special nature of the particular events studied. It may also be said that an understanding of early, as well as later, development depends largely on knowledge of the manner of formation of the egg, that is, of the processes that endow the oocyte, in contrast to other tissue cells, with the capacity to form a new individual. The first paper deals with one aspect of this subject which is also partly considered in some of the others. The next five papers treat principally with the initiation of development, and the remaining seven center primarily about subsequent events as related to nuclear and cytoplasmic factors.

As with most symposia the general purpose of this one was to bring together a group of individuals working in related fields so that there would be opportunity for participants and audience to profit directly from exchange of knowledge and ideas. It was also decided that the presentations be made available to others in published form.

Since the symposium was planned for a one day session, the number of speakers was correspondingly restricted. However, for the purpose of the present volume it was considered desirable that the coverage of the general field be expanded somewhat. This has been done by the inclusion of articles requested from some additional investigators, in this country and abroad, whose work pertains to the subjects under consideration.

It will be readily recognized that the present volume comprises a selected sample, rather than a more comprehensive coverage of investigations into problems of the initial developmental changes undergone by the egg and early embryo. The investigations presented in the various communications cover both descriptive and experimental work on the biological and chemical levels. Many of the articles contain results of previously unpublished researches as well as general reviews of the particular subject.

No special attempt was made to force the contributions into a preconceived plan, or to develop an overall general concept. The interrelationships of the individual topics assured a reasonable amount of unity to the work. In addition, there was exchange of articles among some of the contributors, besides the verbal discussions at the symposium, that provided opportunity for further integration and for elimination of unnecessary repetition. The instances of overlapping that remain were considered desirable, especially where there were differences in outlook and interpretation. Although such differences reflect, in part, variations in point of view and judgment of the individual contributors, they serve mainly to emphasize the lack of critical information concerning the particular problem under discussion. In fact we consider much of the value of a work such as this to reside in the extent to which it brings to the attention of students and investigators these regions of uncertainty and indicates the kinds of problems that are in urgent need of solution, along with the modern methods by which answers may be sought.

Apart from their intrinsic interest and the measure of progress that they provide, the specific discoveries and analyses presented in this book serve, then, to exemplify various approaches toward our understanding of the manner in which sperm and egg contrive to produce a new individual.

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SOME STUDIES ON DIFFERENTIATION AND DEVELOPMENT OF THE OOCYTE *

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When one considers the development of the oocyte, it becomes apparent that there are two general problems to be studied. One of these is the problem of the origin of the egg cell; the other, that of its later development.

The study of the origin of the primary germ cell, from which the oocyte is derived, has provoked wide interest and considerable controversy. Out of this has arisen the following questions: Is there a separate germinal plasm which gives rise to the primordial germ cells, these in turn migrating to the gonad and there forming definitive gonia; or do some or all of the definitive germ cells arise from somatic cells of the germinal epithelium? In some invertebrates it is readily observed that the functional germ cells are derived from a line of cells set apart very early in development, although in the coelenterates and annelids this early setting apart is still questionable (Berrill and Liu, 1948; Wilson, 1928). In vertebrates, and especially mammals, the evidence for the origin of the definitive germ cell from a primary cell type is far from being conclusive. Although there is general agreement that a primordial germ cell type is found, there is disagreement as to whether these cells migrate into the gonad and there eventually differentiate into mature oocytes, or whether they degen-

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erate completely before any definitive cells are formed. In the latter case the oocyte would arise from the germinal epithelium. Some recent studies on this problem include those of Vincent and Dornfeld (1948), Everett (1945), and Jones (1949). An excellent review of the problem of germ cell origin is given by Nelsen (1953).

The other aspect of the general problem of the development of the oocyte is that of its differentiation and growth into the mature egg. For the purposes of this discussion, it is convenient to define three general areas of influence on the developing oocyte. Two of these might be called external, namely, the contributions to the developing egg cell by the surrounding circulatory fluids and the contributions to the oocyte by surrounding cells. The third area is the effects of the oocyte nucleus on the development of its own cytoplasm.

Considerable information is now available regarding the contributions of the circulatory media to the developing egg. Classically, the yolk in avian eggs has been considered to be derived from large molecules carried in the circulation (Romanoff and Romanoff, 1949). Probably the most clear-cut demonstration of the transfer of blood proteins to the egg is that of Telfer (1954). He found an albumin-like, antigenic protein present in very high quantities in the blood of female *Cecropia* during the pupal stage. He was then able to demonstrate conclusively that this protein was transferred to the yolk of developing egg cells. Immunological similarities between the yolk and adult blood proteins have been detected in vertebrates (Nace, 1953), and this suggests that in toto transfer of protein (or at least of specific combining sites) occurs in these forms as well. In his general review of the development of immunological properties, Tyler (1955) pointed out that there is evidence that similarities exist between blood proteins and both the ground substance and the formed elements of the egg cytoplasm. Considerable importance must be attached therefore to the contributions of the circulatory fluids to the development of the oocyte.

The effects of the surrounding cells on the oocyte are varied.

Apparently, they must provide some of the differentiating influences that allow the presumptive oocyte to form an egg, since egg cells are not known to differentiate outside the ovary. A discussion of some of these inducing influences is given by Vincent and Dornfeld (1948). The follicle cells which surround the oocyte are usually considered to function in some positive manner during the growth of the oocyte—either by direct transfer and/or fusion of all or part of the follicle cell to the developing egg, or by acting as a transferring system for substances provided by the blood stream.

In the cases in which the accessory cells contribute directly to the substance of the oocytes, the accessory cells are known as nurse cells. At times, the egg cell and its associated nurse cells can be shown to have been the offspring of a single parent cell (see discussion in Wilson). Often the transfer of not only cytoplasm but also nuclear material is observed in the oocyte-nurse cell relationship. (For recent histochemical studies of such relationships see Schrader and Leuchtenberger, 1952.) It seems reasonable to assume that the "excess" amount of deoxyribonucleic acid found in the cytoplasm of oocytes may arise from such nuclear transfer (Zeuthen and Hoff-Jørgenson, 1952; Marshak, 1953). The nature of the cytoplasmic materials that are transferred to the egg is discussed by Schrader and Leuchtenberger. Where direct transfer of large cellular fragments is concerned, it is obvious that there does exist a mechanism for considerable modification of oocyte development by its cellular environment.

The existence of transferral mechanisms ascribed to the follicle cells is based primarily on inference. Inasmuch as this cellular layer is interposed between the blood stream and the egg, it has generally been assumed that the follicle cell functions in the transfer of materials to the oocyte cytoplasm. Little experimental evidence is available to confirm or deny the validity of such an assumption.

The third area of influence, that of the effects of the oocyte nucleus on its own cytoplasm, has been widely studied. The nuclear changes which accompany the growth of the oocyte are so

striking that they have long been correlated with the cytoplasmic changes which occur. The tremendous enlargement of the nucleus, specialized modifications of chromosomal structure, and the formation of very large or very many nucleoli, have been interpreted as a reflection of nuclear intervention in cytoplasmic differentiation and growth. The many morphological studies of oocyte development have generally led to the conclusion that the nucleoli and the nucleic acid content of the nucleus play a major role in the oocyte. These studies include those of Montgomery (1898), Brachet (1950, 1955), Painter and Taylor (1942), Panijel (1951), and Wittek (1952).

My own cytochemical and biochemical studies on the role of nucleoli and nucleic acids in the development of the egg are primarily concerned with ribonucleic acid (RNA). RNA has been assumed to be related to the synthetic activity of cells since the pioneering studies of Caspersson and co-workers and, independently, Brachet, in the late nineteen thirties (Brachet, 1950; Caspersson, 1950). Both of these workers believed that nuclear RNA was somehow involved in the cytoplasmic expression of genetic activity. This concept has attained new importance in a more specific way in that RNA has been repeatedly suggested as an agent which could receive genetic specificity residing in nuclear deoxyribonucleic acid and transfer this specificity to synthetic centers in the cytoplasm (Dounce, 1953; Rich and Watson, 1954; Goldstein and Plaut, 1955; Lockingen and DeBusk, 1955; Gamow and Yčas, 1955).

In the experiments reviewed below, the starfish oocyte has been used in the study of nuclear RNA. These oocytes can be obtained readily in all stages of development, and in large quantities. They have a large nucleus and nucleolus, the latter of which can be isolated in considerable quantity. In addition, the nucleolus of the starfish oocyte appears to contain all the RNA of the nucleus (Vincent, 1952). By combining qualitative and quantitative histochemistry with direct chemical analyses of isolated nucleoli, it has been possible to study certain aspects of RNA metabolism in the starfish oocyte as it relates to the functional activities of this cell.

Growth of the Starfish Oocyte

In order to relate some of the chemical changes observed in the nucleolus to oocyte growth, it was necessary to determine the size relationships of the nucleolus, nucleus, and cytoplasm of the starfish oocyte. The relationship between nucleus and cytoplasm in the starfish oocyte follows a rigid pattern during growth.

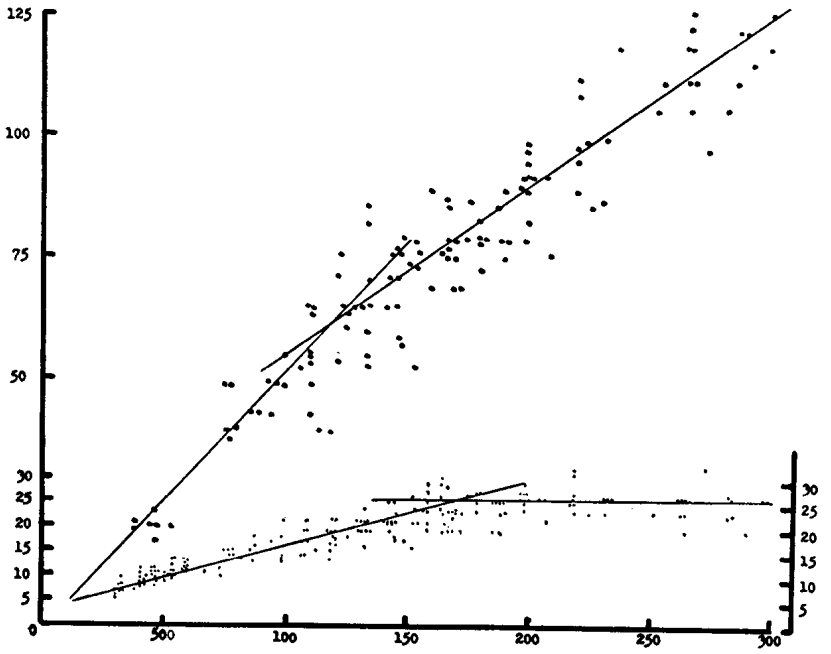

Fig. 1. Diameter of *Asterias rubens* nuclei (large dots) and nucleoli (small dots) plotted against oocyte diameter. Ordinate: diameter of nucleolus or nucleus; abscissa: oocyte diameter in microns.

Since I have been unable to determine an exact time scale for the growth of the oocyte, changes in size of the nucleus and nucleolus are plotted against oocyte diameter (Fig. 1). The relationship shown here seems to be a general one for oocytes, as similar growth curves are found in amphibians (Gall, 1955) and in rats (Vincent, unpublished data). The volume of nucleus and cytoplasm increases proportionately until the oocyte reaches about one-half its mature size (Fig. 2). At this time the rate of cyto-

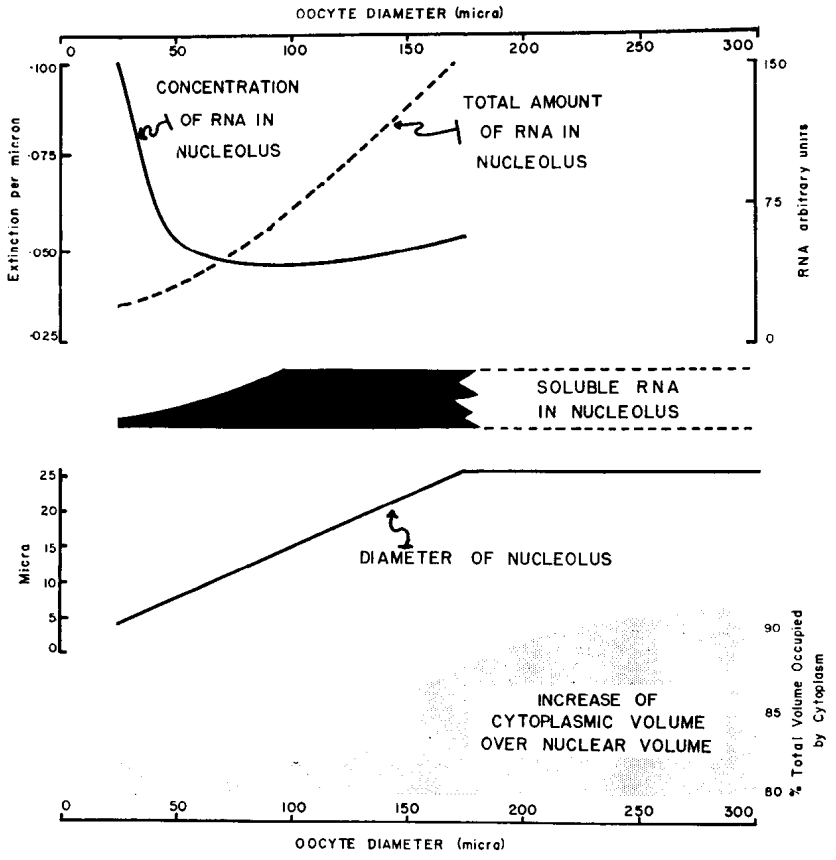

Fig. 2. A comparison of some of the changes in nucleolar RNA and the growth of the oocyte. (Data from Figs. 1 and 3.)

plasmic contribution to cell size increases markedly. This is indicated by the break in the slope of the curve for the nucleus in Fig. 1. The diameters of the nucleolus and the oocyte increase proportionately until the latter attains about one-half its mature size, when all growth ceases (curve for the nucleolus in Fig. 1).

RNA Content of Individual Nucleoli

Some measurements on the RNA content and concentration of individual nucleoli are pertinent in this regard. The ultraviolet absorption of nucleoli isolated in distilled water was compared

with the absorption of nucleoli which had been fixed in formalin prior to measurement. The RNA was then removed with hot perchloric acid and the absorption of nucleoli was remeasured. The latter value was taken as the nonspecific absorption and was subtracted from the total absorption of the nucleolus; the difference was considered to be due to the RNA present. The results are shown in Fig. 3 in which the upper curve indicates the amount of RNA in formalin fixed nucleoli and the lower curve, the values

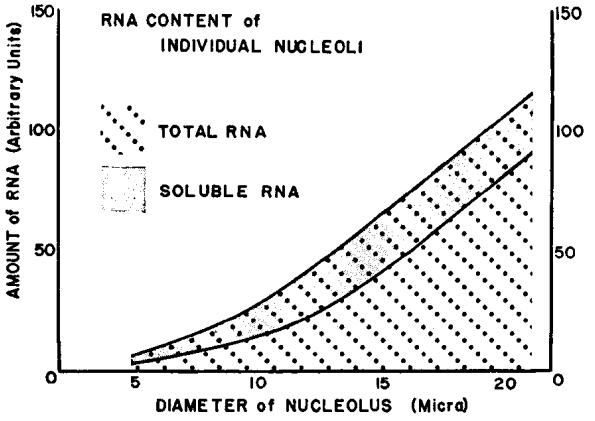

Fig. 3. RNA content of individual *Asterias rubens* nucleoli fixed in formalin (dotted and shaded area) and nucleoli isolated in distilled water (dotted area only). Measurements were made with a microphotometer after Lison (1950) employing Beck reflecting objective and condenser N.A. 0.65, 10× quartz ocular. Light source: 2537 A. line of low pressure mercury lamp.

obtained from nucleoli isolated in distilled water. The difference between the two curves (shaded in the figure) is of interest. First, it indicates that there is less RNA in isolated nucleoli than in fixed nucleoli of the same size. Second, the pattern of difference in RNA content is proportional to the total amount present until the diameter of the nucleolus reaches about 12 microns. Beyond this, the difference between the two remains a constant value. A possible interpretation of this change will be discussed below. The differences in RNA content found in this experiment complement the radiophosphorus studies, suggesting again that starfish

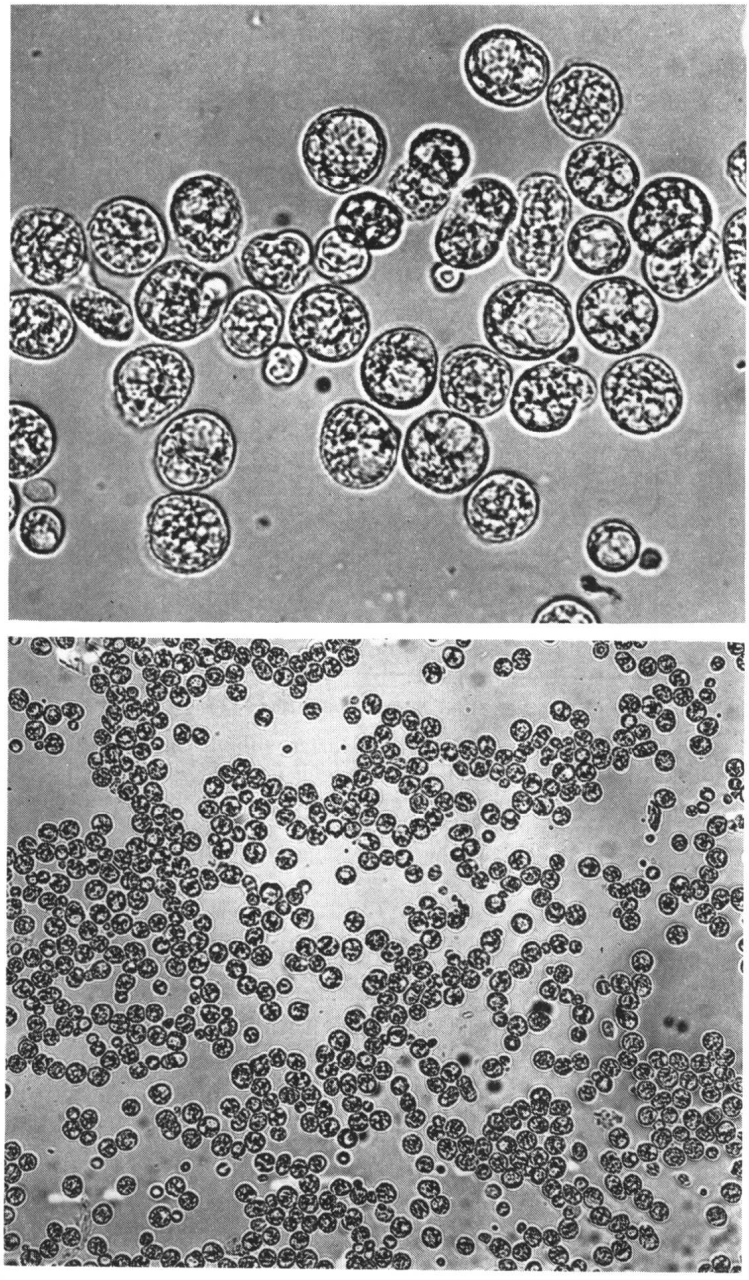

Fig. 4. Photomicrograph of preparation of *Asterias forbesii* nucleoli isolated in Ca-sucrose solution. Nucleoli suspended in 0.34*M* sucrose, visible light illumination. Left, 145×; right, 600×.

nucleoli possess a RNA fraction which is readily soluble in distilled water.

A summary of some of the data on nucleolar RNA is given in Fig. 2. Here the data are plotted against oocyte diameter and related to the changes in cytoplasmic volume which are shown on the lower part of the figure. In general, one finds that the oocyte enters a new phase of activity at about 100 microns, with the onset of rapid cytoplasmic growth. This can be attributed to the synthesis of yolk proteins. This new phase of activity is reflected also in a drop in cytoplasmic RNA concentration not shown here, but readily apparent on slides stained with basic dyes. Such a cytoplasmic picture is typical of oocytes (Vincent and Dornfeld, 1948; Brachet, 1950; Panijel, 1951; Dalcq and Van Egmond, 1953). The nucleolar changes which precede cytoplasmic synthesis are of considerable interest (Fig. 2). In general one finds a shifting from curvilinear to linear relationships. RNA concentration drops very rapidly during early growth of the oocyte, but with the onset of the production of constant amounts of soluble RNA, maintains a constant concentration, although the nucleolus continues to grow. The nucleolus stops growing shortly after the onset of yolk deposition.

RNA Metabolism in Starfish Oocytes

By studying isolated nucleoli it has been possible to obtain some information about the metabolism of RNA in the oocyte nucleus. The advantages of working with pure preparations of isolated cell organelles are well known, and therefore a technique was developed whereby nucleoli could be isolated in quantity from starfish oocytes (Vincent, 1952). Distilled water was used as an isolation medium. Baltus (1954) reported that a modified technique in which sugar solutions of high density were used gave greater biochemical integrity than the distilled water medium. The technique was tedious and the yield low, however. The results and interpretations reported below are based primarily on studies carried out on nucleoli isolated in a Ca-sucrose medium. This process was developed when it became apparent that RNA was lost from the nucleoli into the distilled water iso-