Advances in
MORPHOGENESIS

VOLUME 1

Advances in MORPHOGENESIS

Edited by

M. ABERCROMBIE
Department of Anatomy
University College
London, England

JEAN BRACHET
Faculté des Sciences
Université Libre de Bruxelles
Belgium

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CONTRIBUTORS TO VOLUME I

CH. DEVILLERS, *Laboratoire d'Anatomie et d'Histologie Comparées, Sorbonne, Paris, France.*

R. J. GOSS, *Department of Biology, Brown University, Providence, Rhode Island.*

F. E. LEHMANN, *Zoological Institute, University of Bern, Switzerland.*

G. V. LOPASHOV, *Institute of Animal Morphology, Academy of Sciences of the U.S.S.R., Moscow.*

H. Y. MOHAN RAM, *Department of Botany, University of Delhi, India.*

G. REVERBERI, *Institute of Zoology, Palermo, Italy.*

F. C. STEWARD, *Department of Botany, Cornell University, Ithaca, New York.*

O. G. STROEVA, *Institute of Animal Morphology, Academy of Sciences of the U.S.S.R., Moscow.*

CHARLES E. WILDE, JR., *Department of Pathology, University of Pennsylvania, Philadelphia, Pennsylvania.*

TUNEO YAMADA, *Faculty of Science, Nagoya University, Japan.*

EDGAR ZWILLING, *Department of Biology, Brandeis University, Waltham, Massachusetts.*

PREFACE

The intention of Advances in Morphogenesis is to help to link up the various branches of biology that deal with development. It will seek to do this by discussing topics of current interest, in a form that we hope will be intelligible to a wider circle than the author's immediate colleagues. We regard as at least potentially within its scope any study of the new formation or of the remodelling of living material. The diversity of morphogenesis thus conceived is very great, but not so great that the different branches cannot learn from each other. We may hope that their mutual stimulation will encourage the synthesis of some much-needed general ideas about mechanisms of development which, when successfully framed, will surely begin the next great advance in biology.

The series will have a considerable range of style as well as of content. It is no longer possible to review completely everything that has been published on a biological topic, unless it is a very restricted or exceptionally neglected one. An attempt to achieve the impossible usually ends in a heaping up of data and opinions which obscures any coherent framework of theory. Some way of keeping a wide-ranging discussion in focus is necessary. An increasingly common solution to the problem is to build some or all of a review frankly around the author's own work, discussing this against a background of the relevant research of others. Self-centred reviews of this kind have the additional advantage that scientific workers tend to be considerably more interested in their own research than in anyone else's, and the liveliness of their writing and the acuity of their insight is correlated with their interest. But one-sided chapters can always be balanced by subsequent reviews in the same field, and this form of writing must be welcomed, along with any other variant of the review paper which may help to discourage the proliferation of clones of scientific thought.

J.B.
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CHAPTER I

A CHEMICAL APPROACH TO THE PROBLEM OF THE ORGANIZER

TUNEO YAMADA

I. Introduction

If a vertebrate at the end of its development is represented as a spatial pattern composed of a number of discrete parts, differentiation is the process by which each part is produced out of unspecialized cells which together make up the embryo at an early stage of development. In the later phase of development, various differentiation processes distinct from each other are occurring simultaneously in separate groups of cells within an embryo. The embryonic organization, i.e. the allocation of differentiation processes to various areas of the embryo, is laid down in its general feature by the polarity of the egg cell or by an interplay of factors derived from the egg cell polarity, sometime after fertilization. However, realization of the plan of organization is dependent upon a multitude of epigenetic events occurring in later stages. The action of 'the organizer' is the most important of these events. A series of well-designed surgical experiments demonstrated that the function of the

organizer is to initiate directly or indirectly many of the differentiation processes in other areas of the embryo (induction), and at the same time, to initiate in its own area a number of differentiation processes leading to formation of the essential part of the axial system of the embryo. The effect of the organizer, from the standpoint of the area upon which it acts, is to induce this area to switch from one type of differentiation to another, but not to acquire the ability to differentiate. In spite of this fact, since the type of differentiation induced by the organizer leads to the formation of the central nervous system, the importance of the organizer in vertebrate development is unquestionable.

Several years after the discovery of the organizer action by Spemann and H. Mangold (1924), a vivid interest arose in the chemical nature of the factor responsible for embryonic induction. Two prominent groups of workers, the Cambridge group including J. Needham, Waddington and others, and the Freiburg group including H. G. Fischer, Wehmeier, H. Lehmann and others, conducted extensive research in the field. Although the results were impressive and soon followed by the experiments of Barth, Suomalaien and Toivonen, Okada, and others, a pessimistic atmosphere became prevalent, owing to the conviction that what had been found by these researches seemed to be the activating principle (evocator) which needs the organizing influence (individuation) from the living embryo to bring about the formation of the secondary embryonic axis.

In the forties, Brachet attacked the problem from a new angle, and obtained results strongly supporting his view that RNA is the basic factor in embryonic induction and differentiation. Meanwhile another line of investigation, headed by Toivonen and Kuusi, attempting to identify factors responsible for regional induction caused by adult mammalian tissues was making its laborious beginning. In the last decade, the ever increasing interest in cytochemical and biochemical aspects of growth and differentiation, together with progress in biological techniques, has given new impetus to the investigation into the problem of embryonic induction.

Various new approaches are now being carried out, broadening the scope of the problem and in the present articles some of these recent advances will be discussed together with new data accumulated in our laboratory. An attempt will be made to integrate the data obtained in the various types of approach. Readers not acquainted with embryological aspects of the subject are referred to the following publications: Weiss (1935); Spemann (1936); Lehmann (1945); Brachet (1947b, 1957); Pasquini (1949); Needham (1950); Pasteels (1951); Raven (1954); Holtfreter and Hamburger (1955); Boyd (1955); Waddington (1956); v. Woellwarth (1956); Dalcq (1957b).

To avoid misunderstanding, it may be convenient to clarify the use of a few terms employed repeatedly in the present article. The term organizer is used according to Spemann (1936) to denote a living embryonic area or a piece of it endowed with the special faculties suggested above, but not to denote a chemical or physical entity responsible for the organizer action, for which expressions such as agent or factor are reserved. The word inductor indicates any living or non-living system which has an inducing effect on a competent reacting system. Embryonic induction or simply induction is used whenever the mode of differentiation of a developing system is obviously changed under the influence of another developing system or of a factor coming from the outside. The word is used regardless of whether the outcome of differentiation of the reacting system is simple or complex, organized or unorganized, and whether the underlying mechanism conforms with that of normal induction or not. Words such as evocation, individuation, and dorsalization are used according to the meanings defined by their proposers.

II. Fine Structure of Embryonic Cells as Revealed by Electron Micrographs

It is rather surprising that until quite recently many developmental phenomena, particularly embryonic induction in vertebrate embryos, have been discussed without essential knowledge of subcellular components of the cells which are participating in the phenomenon. This was chiefly due to the fact that the embryonic cells of vertebrates is a difficult subject for the methods of classical cytology. The introduction of cytochemical methods helped to clarify many of the problems, but it is undeniable that these methods have their limitations. Only with electron microscopic study of thin sections has it become possible to identify and characterize the essential morphological components of these cells. In this section, subcellular components of the amphibian embryonic cells which participate in the organizer action will be described, and their possible role in induction and differentiation will be discussed.

According to the electron microscopic studies of Eakin and Lehmann (1957), and Karasaki (1959a), the subcellular components recognized in the cytoplasm of the amphibian embryonic cell, during the early phase of development are as follows: Mitochondria, lipochondria (lipoid droplets), micro-particles, cytoplasmic vesicles (endoplasmic reticulum), pigment granules, Golgi complex, yolk platelets, and cell membrane.

A. Mitochondria

The mitochondrial population in the ectodermal cell, which has been studied most carefully, reveals a progressive maturation in morphology

during the early phase of development. In *Triturus pyrrhogaster*, mitochondria of the presumptive ectoderm cells of the earliest gastrula are represented by spheroids or ovoids with diameter varying from 0·3 to 0·7 μ. Cristae are few and only incompletely formed. At this stage and earlier, beside mitochondria a number of smaller darker spheroids without internal septa are observed and provisionally called dense bodies. According to Karasaki they may represent the precursor of mitochondria. In the late gastrula of *Triturus*, when the presumptive ectoderm is underlain by the organizer layer, the presumptive neural cells show rod-shaped or long oval mitochondria as well as spherical ones. In the rod-shaped mitochondria, cristae are conspicuously formed but in small numbers, leaving a wide space between individual cristae. Later in the neurula, the mitochondria of the neural plate cells are larger in number and size compared with those of the preceding stages. Many filamentous mitochondria furnished internally with numerous cristae are present. The tendency to structural elaboration is further accentuated in the cells of the wall of the spinal cord of the tail-bud stage. Here, mitochondria as long as 6 μ can be seen often forming a dense group. The matrix of the mitochondrion is denser than earlier, and a complicated system of cristae fills up the interior. According to Eakin and Lehmann (1957) a similar transformation also occurs in the mitochondrial population in the neural cell-line in the embryo of *Xenopus laevis*.

In *Triturus*, the change in the mitochondrial population in number and structure found in cells of the epidermal region during gastrulation and neurulation is less pronounced than that in the neural region (Karasaki, 1959a).

As it is very probable that the enzymatic activities of a mitochondrion are dependent upon the area of its membranes, especially of cristae, the conclusion that the morphological elaboration of the mitochondrial population observed in the neural cell-line during the phase of induction and differentiation reflects the rise in the biochemical activity of the population appears unavoidable. In this respect, the findings of Boell and Weber (1955) are important; they isolated the mitochondrial fraction from the *Xenopus* embryo and found an appreciable increase in its cytochrome oxidase activity (per h per μg N) during early development.

According to unpublished data of Karasaki, the cells of the invaginating organizer area (the dorsal mesoderm) of the earliest gastrula of *Triturus* contain beside spheroidal or ellipsoidal mitochondria, a number of elongated mitochondria with cristae, which are almost absent in the ectodermal cells of the same stage (Figs. 1 and 2). On the other hand, the cells of the ventral mesoderm, which is not yet invaginating at this stage, do not show such elongated mitochondria. This finding, together

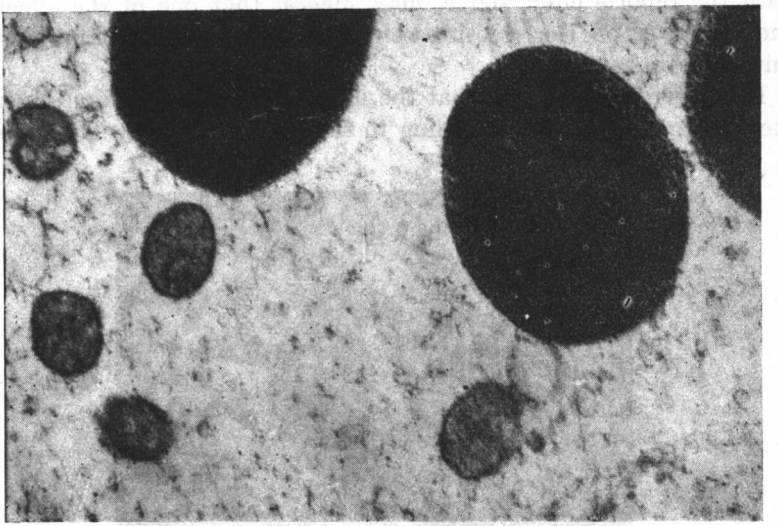

FIG. 1. A part of the ectoderm cell of a middle gastrula of *Triturus pyrrhogaster* showing spherical mitochondria, yolk platelets provided with external layers, and the loose mesh work of micro-particles. × 25,000 (Karasaki).

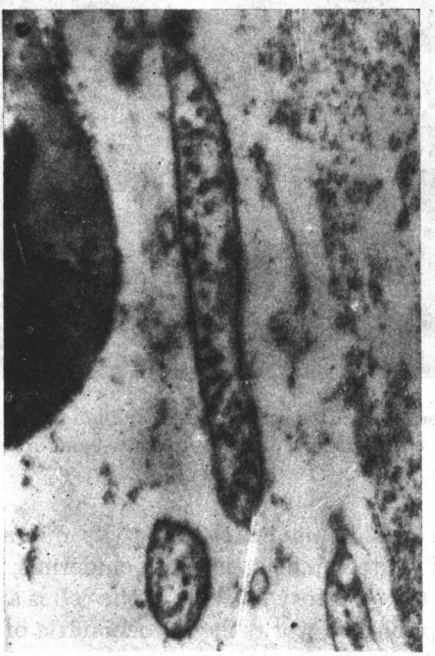

FIG. 2. Slender mitochondria of an organizer cell after invagination through the blastopore of a middle gastrula of *Triturus pyrrhogaster*. × 25,000 (Karasaki).

with the reports cited above, may suggest that one of the effects of the organizer on the reacting ectoderm cells is the enhancement of mitochondrial activity.

It is interesting to note that the mitochondrial population differs in morphological features according to the embryonic areas. In *Xenopus*

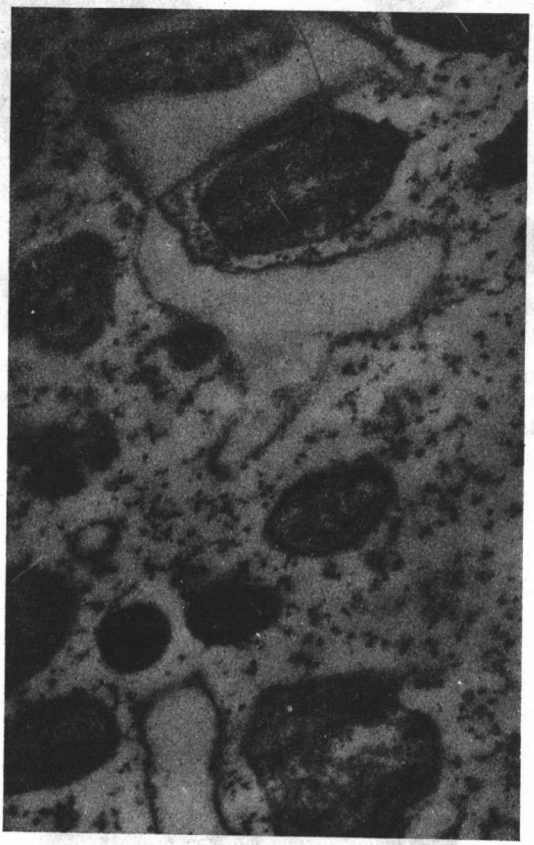

FIG. 3. An ectoderm cell of the early gastrula of *Bufo vulgaris*. Free micro-particles, mitochondria with clear cristae, pigment granules, and irregular cell membranes. ×40,000 (Karasaki).

embryos, Eakin and Lehmann (1957) point out a special form and arrangement of mitochondria in the epidermis, whose functional connection with the secretory activity of the cell is apparent. According to Karasaki (unpublished work) the mitochondria of the epidermis cells of the *Rana* embryo also can clearly be distinguished from the mitochondria of the neural cells.

B. Ribonucleoprotein Particles and Cytoplasmic Vesicles

According to Karasaki (1959a and unpublished data) dense particles having an average diameter of *ca.* 180 Å are scattered freely or in clusters of several particles in the cytoplasm of early embryonic cells of *Triturus pyrrhogaster*, *Bufo vulgaris* (Fig. 3), and *Rana japonica*. At the tail-bud stage, although most of the particles are still found free or in small clusters, some of the fine particles (micro-particles) are found attached to the surface of cytoplasmic vesicles which have increased in number at this stage. From the high RNA content of the ultracentrifugally prepared fraction of the fine particles (36% RNA) they may be identified as ribonucleoprotein particles or Palade granules. There apparently occurs an increase in the frequency of the fine particles in the neural cell-line during the period between the gastrula and the tail-bud stages. If we assume that the bulk of ribonucleoprotein of embryonic cells is in the form of fine particles such an increase is to be expected from the increase in the RNA content of the total embryo, as demonstrated by Steinert (1951) and of the neural area, as suggested with histochemical methods by Brachet (1947a, b, 1957) or with chemical analysis by Takata (1953). Also in embryonic cells of the early chick embryo similar fine particles are shown in the electron micrographs of Bellairs (1958, 1959).

Coming back to amphibian cells at the early gastrula stage, the membranous structure of the endoplasmic reticulum system is represented only by small spherical or ovoidal vesicles, devoid of the fine particles (smooth-surfaced). At this stage, these cytoplasmic vesicles are very infrequent in *Triturus* ectoderm cells while somewhat more frequent in *Rana* and *Bufo* ectoderm cells (Karasaki, 1959a and unpublished data). An increase in the frequency and size of these vesicles with the progress of development is clearly indicated. At the tail-bud stage, many of the cytoplasmic vesicles are furnished with fine particles attached on the external surface (rough-surfaced). In this case also, membranes assume the form of a sphere, ellipsoid, or short tubule, but never that of packed lamellae, although in the larval tissue of Amphibia one often finds the condition of closely packed double lamellae. The rough-surfaced cytoplasmic vesicles can be observed in the epidermal cells as well as in the neural cells. According to Bellairs (1958, 1959) the rough-surfaced endoplasmic reticulum in the chick embryo is present already at the primitive streak stage, though in small amount. She made a quantitative estimation of the frequency of the rough-surfaced endoplasmic reticulum per unit area of the section in the neural cell-line, and found a strong increase during the period from the long primitive streak stage to 10 days of incubation, in which the whole