Cell Surface and Differentiation

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Preface

Cell surface membranes contain diverse kinds of molecules, notably proteins. They play critical roles in the regulation of cellular activities such as growth and differentiation. Knowledge of the structure and function of cell surface molecules has increased enormously in recent years, partly because of recombinant DNA techniques.

This book describes the current state of a fast growing research area, namely the molecular biology of the cell surface with respect to cell differentiation. The role of cell surface molecules in differentiation is the central subject of this book, but I have also dealt with cell surface markers, which are useful in monitoring differentiation, and cell adhesion molecules, which influence differentiation. Since the book is multidisciplinary in nature and the readers are expected to include students and graduate students, I have included two introductory chapters in order to familiarize readers with current developmental biology and molecular studies of the cell surface. Although the scope covered by this book is large, I tried to keep it concise, so that an interested reader can read through it in a few days.

I am grateful to Dr Paul H. Atkinson, who offered helpful criticisms and suggestions regarding the content, to Dr Susan Hemmings of Chapman and Hall for hearty co-operation and to Miss Kumiko Sato for all the secretarial work needed to prepare the manuscript.

Contents

1 Biological systems

A fertilized egg gives rise to many different cell types, such as nerve cells, myoblasts, cartilage cells and endothelial cells. The whole or a part of the process where a cell yields two or more cell types is called differentiation [1]. The elucidation of the mechanism of differentiation is one of the most fascinating studies in biological sciences. Differentiation is most often observed during embryogenesis; but even in adulthood certain cells, such as blood cells and the epithelial cells of the small intestine, differentiate continuously from stem cells. Important concepts in classical embryology were formulated from studies using the sea urchin, newt and chicken. The processes of development in these animals are relatively easy to observe. Furthermore, the large size of the newt and chick embryos have allowed experimental manipulation of the developmental processes by surgical procedure. However, the current preferred systems study the molecular mechanism of embryogenesis, and are usually the fruit fly, nematode and mouse, although the use of amphibians is being revived in exciting experiments in frog embryos. Undoubtedly, organisms in which a genetic approach is possible are much preferred. This introductory chapter deals with the biological systems used frequently in developmental biology and some of the concepts of the discipline.

1.1 NEMATODES AND CELL LINEAGE

The formation of a differentiated cell from a fertilized egg consists of many steps. Several intermediate cells arise before the final appearance of a differentiated cell, and the pathway of differentiation is called cell lineage. A description of cell lineage is essential for a precise understanding of differentiation. In each step of cell lineage, namely from one cell to the next cell, the following questions must be answered:

1. Is the differentiation pathway one-way, bi-directional or multi-directional?
2. Does the step proceed autonomously or is it influenced by an external signal?
3. If influenced, what is the external signal?

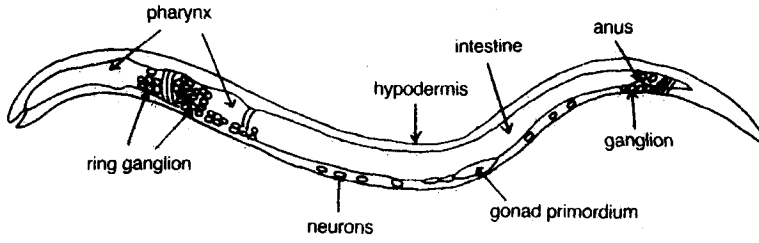

Figure 1.1 A larva of *Caenorhabditis elegans* (based on [2]).

The cell lineage occurring to form a complete organism has been determined only in one organism, the nematode *Caenorhabditis elegans* (Figure 1.1) [2–4]. This worm develops quickly – the 560-cell embryo hatches 14 hours after fertilization. About a day after hatching, it becomes a mature worm which is 1 mm in length and has 959 somatic cells. The cell lineage was determined by observing the fate of individual cells by a microscope, and an outline is described in Figure 1.2. After the first division, a fertilized egg produces two asymmetrical cells designated AB and P_1. The majority of the progeny of AB go on to form dermis and nerve cells. From P_1, EMS and P_2 cells are formed, and EMS produces MS and E. The progeny of E are destined to be intestinal cells, while MS produces many cell types as shown in Figure 1.3. P_2 forms P_3 and C; P_3 produces P_4 and D.

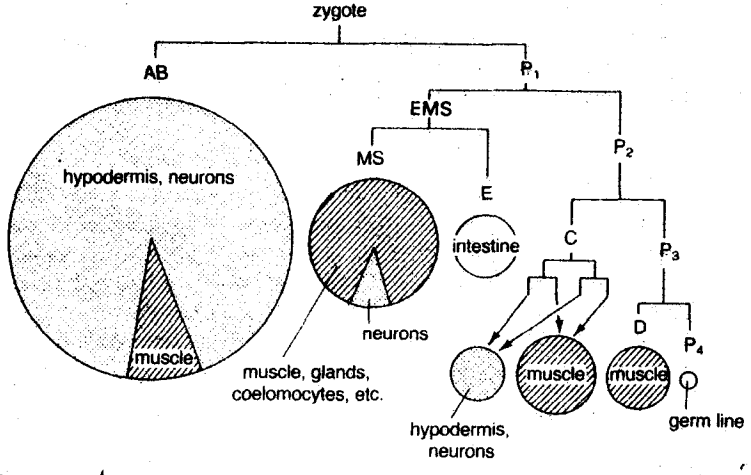

Figure 1.2 Summary of cell-types derived from blastomeres produced by early cleavage. Areas of circles and sectors are proportional to number of cells (cited from [2]). © Academic Press.

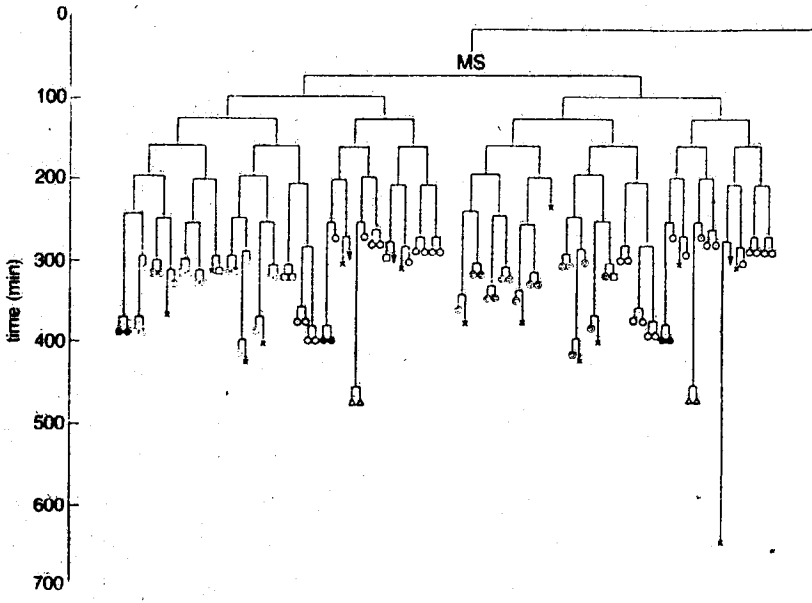

Figure 1.3 A part of cell lineage of *C. elegans* (progeny of MS cell). ● neuron; ◎ pharynx cells; ○ muscle cells; △ coelomocytes; □ other cells, unidentified cells; × death; ➤ cells that divide in larval development (cited from [2]).

P_4 forms only germ cells, while D is devoted to form only muscle. It is notable that the cell lineage which forms even a tiny worm is very complex.

When a cell in the intermediate stage of differentiation was selectively killed by a lazor beam, the developmental fate of other cells was largely unaffected. Thus, the cell lineage of *C. elegans* was considered to be 'rigid'. Fertilized eggs are often classified into mosaic and regulative types [1]. In the mosaic type the fate of blastomeres created by initial cell divisions is largely predetermined, while in the regulative type the fates of the initial blastomeres are not determined. In view of the rigid lineage, the egg of *C. elegans* is a typical example of a mosaic type. The observation that cell fate is predetermined for each blastomere of a mosaic egg implies that a certain cytoplasmic determinant of cell differentiation is present in the egg cytoplasm. The unequal distribution of the determinant which results from cell division plays an important role in the embryogenesis of mosaic eggs.

However, even in *C. elegans*, the differentiation of certain cells does not proceed independently of interactions with other cells. A typical example is muscle formation [5]. In the normal embryo, the progenies of both the AB

blastomere and the P_1 blastomere produce muscle. When the P_1 blastomere is isolated and is allowed to develop in the absence of the AB blastomere, the progeny cells are able to produce muscle. However, progenies of isolated AB blastomere cannot produce muscle. Further analysis has revealed that interaction with the progeny of the EMS cell is required for the progeny of the AB blastomere to produce muscle. Furthermore, when the position of ABa and ABp, which are daughter cells of AB, are exchanged, the progenies of ABa develop as if they are the progenies of ABp cell and vice versa [5].

1.2 MOUSE EMBRYOGENESIS AND ITS MANIPULATION

The mouse is most often chosen in the study of the mechanism of mammalian embryogenesis because the duration of its embryogenesis is short, namely 19–20 days, and the small animal is easy to handle. In addition, there are many useful inbred strains available, especially some mutant strains which are abnormal in certain developmental processes.

The earlier stages of mouse embryogenesis are summarized in Figure 1.4. The fertilized egg divides about 18 hours after fertilization, and subsequent

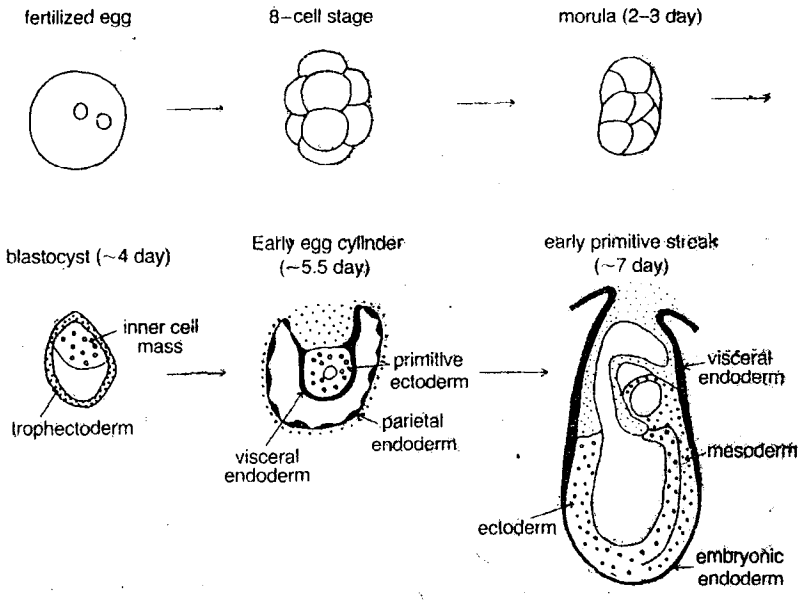

Figure 1.4 Early embryogenesis of the mouse (partly based on [6–8]).

divisions take place every 12 hours during the preimplantation stage. Until the 8-cell stage, each blastomere in an embryo looks identical. At the late 8-cell stage, cell adhesion between blastomeres increases, and the embryo becomes more compact; this phenomenon is called compaction. The embryo at the 16-cell stage is called a morula. At the 32-cell stage two cell groups – externally located trophectoderm and internally located inner cell mass – can be distinguished clearly. The embryo develops a blastocoel and is called a blastocyst. Trophectoderm cells constitute placenta and parietal yolk sac, and the fetus develops from the inner cell mass. At the late blastocyst stage, the embryo implants into the uterine wall.

During implantation, a layer of primitive endoderm cells develops on the surface of the inner cell mass, and the inner cells become primitive ectoderm cells. Primitive endoderm cells differentiate into two types of extraembryonic endoderm cells, namely visceral endoderm cells, which cover the embryo proper, and parietal endoderm cells, which underlay trophectoderm and secrete components of a kind of basement membrane called Reichert membrane. Around day 7 and 8 of embryogenesis, ectoderm cells, mesoderm cells and endoderm cells differentiate from primitive ectoderm cells. On Day 9, the embryo already shows the characteristic features of a vertebrate embryo. Cell layers then interact with each other and form organs.

During embryogenesis many cells migrate for long distances. For example, precursor cells to blood cells migrate from the yolk sac to the thymus, liver and bone marrow; and primordial germ cells, which are destined to be germ cells, migrate from the base of the allantois to the hind gut, and then to the gonads. A detailed description of mouse embryogenesis is found in references [6]; [7] and [8].

Mouse embryogenesis is a typical example of the regulative type: When a blastomere at the 2-cell stage or 4-cell stage was destroyed by a fine needle, other blastomeres compensated for the destruction, and normal embryos developed from the treated ones [9]. A single 8-cell blastomere was also shown to be developmentally totipotent, namely it constituted both embryonic and extraembryonic tissues. Positional information appears to determine the fate of a blastomere at this stage and one of the two differentiation pathways (trophectoderm cells and cells of the inner cell mass) is selected. A blastomere located externally at the 16-cell stage differentiates to trophectoderm cells, while a blastomere located internally differentiates to inner cell mass cells. Indeed, two different cell populations are found at the 16-cell stage; externally located large cells and internally located small cells. The origin of the two distinct cell populations can be traced back to the late 8-cell stage [10]. At that time, asymmetry can be detected on the plasma membrane. In the membrane facing the external surface, microvilli develop and several cell-surface proteins are

concentrated on this side. This phenomenon is called polarization. When cell division occurs parallel to the polarized area, two different cells, namely a polarized large cell and an apolar small cell are generated (Figure 1.5). These observations might indicate that a polarized distribution of cell surface components is associated with the determination of cell fate. However, aggregates composed of a pure population of the large cells as well as those composed of a pure population of small cells can yield normal embryos [11]. Cells of the 16-cell stage might have selected a developmental programme (to yield either trophectoderm cells or inner cell mass cells) but the decision can be altered if there is a drastic change in the environment.

Positional information also appears to be of critical importance in the differentiation of inner cell mass cells into primitive ectoderm cells and primitive endoderm cells. Cells exposed at the surface of the inner cell mass are likely to be triggered to differentiate into primitive endoderm cells.

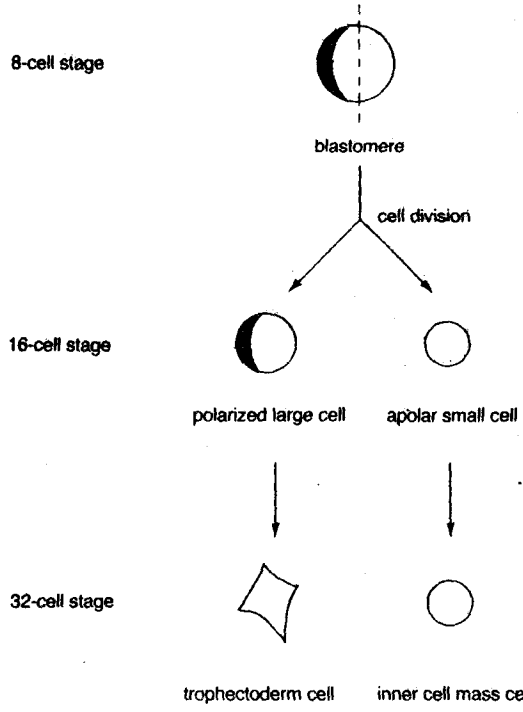

Figure 1.5 Polarization of cell surface molecules and differentiation of trophectoderm cells and cells of inner cell mass.

The flexibility in mouse embryogenesis is also illustrated by the formation of chimeric mice. When two morulae are aggregated, embryos with twice the number of cells adjust themselves and yield mice of normal size and characteristics [12]. The chimeric nature of the progeny can be demonstrated clearly by the mosaicism of the coat colour observed when embryos are used from mice which differ genetically in their coat colour.

The mouse is frequently used in experiments to introduce and express foreign genes in a multicellular organism. For that purpose, exogenous DNA is microinjected into the male pronucleus of the fertilized egg [13]. In the fertilized egg, there are two pronuclei, one derived from the sperm (male pronucleus) and the other from the egg (female pronucleus). Genes are introduced into the male pronucleus, which is bigger than the female one and is more suitable for injection experiments. The fertilized egg with injected DNA is either directly grafted into the oviduct of a foster mouse or cultured *in vitro* to the blastocyst stage and grafted to the uterus of the foster mother. Mice which are subsequently born with integrated foreign DNA are called transgenic mice. Thus, introduction of a growth hormone gene with a strong promoter resulted in the production of a super mouse which had around 1.5 times the body weight of ordinary mice [14]. This result demonstrated the effectiveness of transgenic techniques.

Microinjection is not the only way to generate transgenic mice. Even, the usage of sperm as a vector of foreign DNA was once proposed [15], but the result has not been confirmed so far.

The ability to produce transgenic mice is expected to be helpful in elucidating the role of various molecules during differentiation and development. The most successful approach so far is to introduce a gene with a strengthened promoter, so that the gene becomes expressed at an inappropriate time and place. Abnormalities observed in the resulting transgenic mice give information on the function of the gene product [16]. Inhibition of gene function by the introduction of an artifical gene that produces anti-sense RNA of the target gene is also a promising approach. Indeed, a transgenic mouse with the abnormal nervous behaviour characteristic of shiverer disease was produced by introducing an anti-sense gene of myelin basic protein [17]. Homologous recombination to delete a target gene may also be a technique with general applications, although the technique is still under development [18]. Homologous recombination is performed in embryonic stem cells (ES cells) (section 1.5) to delete a gene in a chromosome, and chimeric mice are produced by injecting the ES cell into blastocysts. By mating the resulting mice, a mouse lacking the target gene both in the maternally derived chromosome and in the paternally derived chromosome is expected to arise.

Nuclear transplantation experiments can also be performed in mouse embryos. Male pronuclei and female pronuclei are removed from fertilized

eggs with the aid of micropipets, and foreign nuclei are introduced by a fusion technique [19]. In such a way, a nucleus from a 16-cell embryo was found to direct the production of a mouse in the cytoplasm of a fertilized egg. However, it became difficult to form a mouse from nuclei from embryos of more advanced stages. It is known that a feeding tadpole can be formed when the nucleus of an unfertilized frog egg is replaced by a nucleus from adult frog cells such as keratynocytes. Thus, the developmental potential of nuclei appears to be profoundly different between the mouse and the frog. Even more surprising is the fact that in an egg whose pronuclei are reconstituted by transplantation, both the male pronucleus and female pronucleus are required to form a mouse – two male pronuclei or two female pronuclei are ineffective [20]. Therefore, it has been concluded that DNA (or chromosomes) in the sperm and the egg are modified in different manners and both types of DNA (or chromosomes) are needed to accomplish embryogenesis.

1.3 EARLY EMBRYOGENESIS OF AMPHIBIANS

Amphibian embryos have been the subject of experimental embryology for a long time, and are still helpful in studying the mechanism of the early stages of cell differentiation. When the early embryo of a newt was lassoed by a hair in the plane where the first cleavage furrow was expected, the two separated portions developed normally and formed twin newts [1]. This result thus clearly indicated the flexibility of the newt embryo. However, when the embryo was divided perpendicularly to the plane of the first cleavage, one half of the embryo formed the intact newt, while the other half yielded just a cell aggregate. Further studies by Hans Spemann have led to a conclusion that embryos with a middle portion called a grey crescent, which is seen in the region opposite the sperm-entry site, can develop into normal newts [1]. The former way of embryo cleavage usually resulted in a partition of the grey crescent into two portions. Therefore, an unequal distribution of a cytoplasmic component in the grey crescent region plays a decisive role in embryogenesis. Furthermore, Spemann and Mangold have suggested an inductive role for the dorsal lip, which is derived from the grey crescent. After gastrulation, the dorsal lip becomes part of the mesoderm, which is known as the 'organizer'. When the ectoderm was underlayed with the organizer, it developed into the neural tube. Otherwise, the ectoderm developed into the epidermis. Thus, the organizer apparently determines the fate of ectoderm cells. Various attempts have been made to isolate the chemical entity of the organizer but these have been unsuccessful. Subsequently, apparently non-specific stimulation, such as an alteration of the pH or ionic strength was found to induce neural differentiation in the ectoderm. Whether the signal delivered by the organizer is also non-specific,

or it is specific and the non-specific stimulation just mimics or by-passes the action of the organizer is not known.

Recent studies have been conducted using the frog *Xenopus laevis*, which is easier to handle and has been yielding important results [21, 22]. When a *Xenopus* embryo was cut horizontally, and the separated pieces were cultured independently, the animal cap developed into the ectoderm and the vegetal portion into the endoderm, while no mesoderm was formed. When the two pieces were placed in contact, the mesoderm differentiated in the lower part of the animal cap. Thus, the vegetal portion has the capacity to induce mesoderm in ectoderm. Further studies recently carried out revealed the importance of growth factors in the induction process (Chapter 4).

As mentioned above, amphibian embryogenesis is a suitable system to show how cytoplasmic information controls embryogenesis. The asymmetry of the animal pole and the vegetal pole is sufficient to yield the ectoderm and the endoderm, and the interaction of the two embryonic portions yields the mesoderm. The dorsal–ventral axis of the embryo is determined by the point of sperm entry.

1.4 DEVELOPMENTAL GENETICS OF *DROSOPHILA*

The fruit fly *Drosophila melanogaster* has been used frequently in the molecular analysis of development [4, 23]. The adult fly is formed from a fertilized egg in only 9 days. In the giant polytene chromosomes found in the salivary glands of the larvae, about one thousand DNA strands and proteins assemble side by side and form characteristic and reproducible banding patterns. There are about 5 000 bands, and the average of each visible band is about 25 kb, and the size corresponds roughly to a gene. When a gene is mutated, especially deleted, one of the bands is usually altered. With this information, together with the result of crossing over experiments, the chromosomal position of the mutated gene can be determined accurately. Many mutants with certain abnormalities in developmental programs have been isolated using this organism. The advancement of recombinant DNA technology has made it possible to clone the gene defining the mutated phenotype by microdissecting the polytene chromosomes or by chromosome walking from the nearest known DNA segments. Chromosome walking is a procedure used to clone a gene, using a DNA probe whose sequence is located near to the desired gene. The identity of the cloned gene can be confirmed by rescuing the mutant phenotype through P-element-mediated transfer of the wild-type gene. Using these approaches, a number of genes specifying developmental steps have been cloned and sequenced. The structure of gene products predicted by the sequence data is providing new and deep insights into the mechanism of differentiation and development.

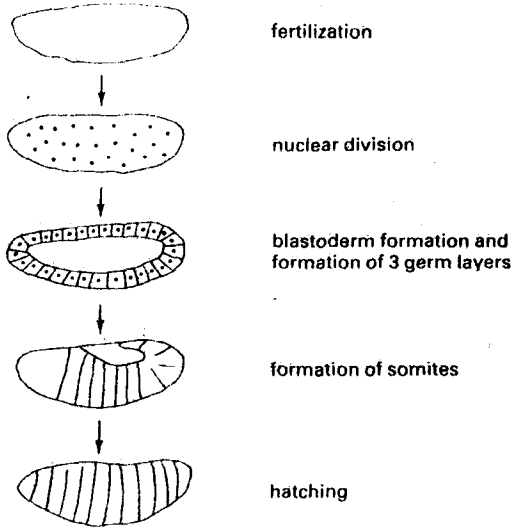

Figure 1.6 An outline of the embryogenesis of *D. melanogaster*.

The embryogenesis of *D. melanogaster* is illustrated in Figure 1.6. After fertilization, nuclear division occurs rapidly. However cell membranes are not formed at the initial stage of embryogenesis. About 1½ hours after the egg is laid, when the nuclei have divided 9 times, nuclei migrate to the surface of the egg. Then the nuclei divide another 4 times, and cell membranes are formed suddenly, resulting in the formation of a cellular blastoderm. At this stage, two embryonic axes are established; they are the anterior–posterior axis and the dorsal–ventral axis. Substances in the egg cytoplasm play important roles in establishing the embryonic axes: some of the maternal factors are asymmetrically distributed in the embryo and this asymmetry contributes to the establishment of the embryonic axes (Chapter 7). Shortly after the emergence of cellular blastoderm, three germ layers are formed, and gastrulation follows. The embryo is then divided into segments, and the embryo then hatches – a day after fertilization.

1.5 DIFFERENTIATION OF TERATOCARCINOMA STEM CELLS

Cells which can differentiate *in vitro* are invaluable in studying the molecular mechanism of cell differentiation. Teratocarcinoma stem cells and