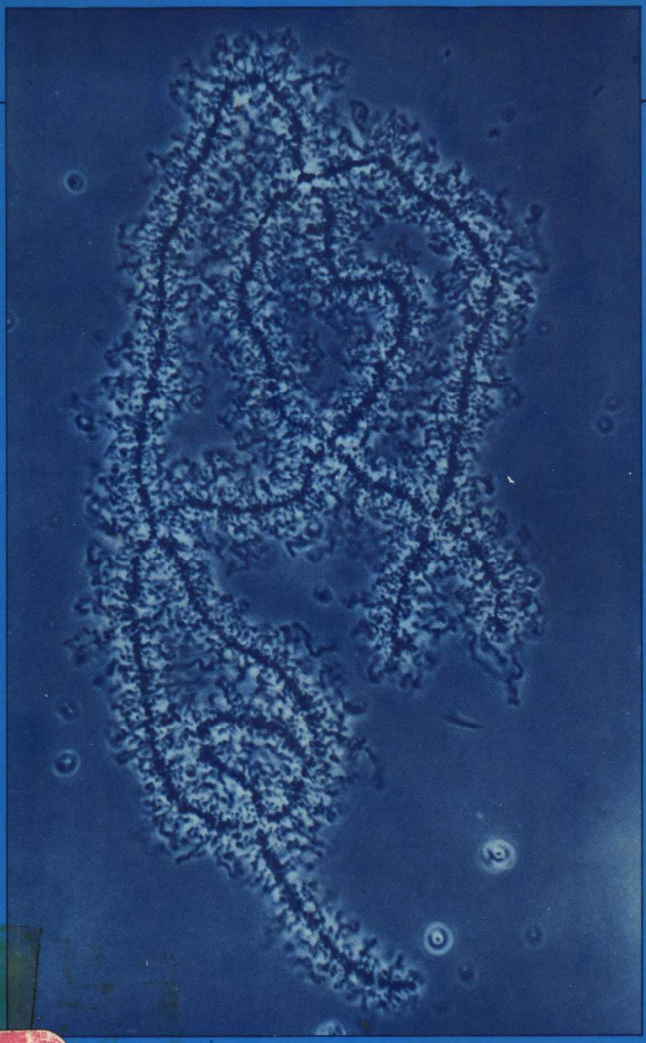
The Differentiation of Cells

Norman Maclean



Genetics—
Principles and Perspectives I

The Differentiation of Cells

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To Margaret, Lorna and Gavin,
with affection

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Preface

This book is intended to provide an introduction to one of the most exciting problems known to man—the differentiation of living cells. How do the diverse types of cells in the same organism, all arising from the same fertilized egg cell, come to be so different? This question, put casually by an unsuspecting lecturer in my undergraduate days, has intrigued me ever since. I hope that this book may help to arouse in some of its readers a similar interest and curiosity.

I have been deeply conscious, while writing the book, of constant oversimplification. Hardly one experiment or observation which has been cited is, on reading the original publication, as definitive or uncomplicated as it has been made to appear. But that is, I suppose, the nature of science. Certainly attempting a synthesis in a general area such as differentiation is somewhat analogous to building a house with bricks all made from different materials from very different localities. That is, pieces of evidence which are put side by side in order to yield a model may be drawn from very dissimilar systems, the one a virus-infected liver cell perhaps, and the other a marine alga. And as I have remarked elsewhere in the text, the areas in which theorizing is easiest but least profitable are those which are very poorly understood. It seems to me that biological science has now moved to a stage where a synthesis of differentiation can be usefully attempted. Thus the book. But since this is a fairly recent development, it would be still very easy to draw inappropriate conclusions, or to use quite irrelevant evidence.

The other difficulty has been a temporal one. I have written this book over a period of two years and much has changed in that time in the field of cellular genetics. Despite occasional revisions, certain parts of the text will be out of date on publication. But most, I trust, will have a slightly longer useful life.

Acknowledgments

I am greatly indebted to past and present colleagues at Southampton, with whom it has been a pleasure to work. Some of them have helped particularly by reading and criticizing parts of the manuscript of this book, especially Dr. Muriel Ord, Dr. David Garrod and Dr. David Morris. Others elsewhere have also provided useful comment and criticism—Dr. David Malcolm, Dr. Godfrey Hewitt and Professor Herbert Macgregor, and particularly the general editors of this series, Dr. K. R. Lewis and Professor Bernard John. To all of these I owe a great debt. I must also thank Mrs. Anne Wharmby for undertaking much of the typing.

Southampton 1976 N.M.

Contents

Introduction: the problem and its importance

There are two biological phenomena which, by reason of their familiarity, are too often taken for granted. Yet they have puzzled and tantalized scientific minds for over a century and we are still far from a proper understanding of either. I refer to the existence of organisms in the discrete categories of species, and the organization of living cells into the distinct groups which we call tissues. The first problem we must leave aside to Darwin and his successors, devoting ourselves in this book to the second problem, an understanding of differentiation.

When a fertilized egg develops into a plant or an animal it does not simply produce a multicellular mass of identical cells. Instead it gives rise to an *organism*, an organized assembly of distinct cell types, the differing cells occurring, for the most part, in discrete tissues. The mystery of the process of differentiation considerably deepened with the realization that differing cells in the same organism all possess an identical and complete set of the genetic material. This discovery ruled out the possibility that differentiation was accomplished by dividing up the genetic material of the fertilized egg into separate portions appropriate to the different tissues. It is clear that, in general, differentiated cells retain a complete set of genes but use only some of them.

We must be aware, however, of the beguiling nature of the theory of selective gene activity in differentiation. For although it affords an understanding of how differentiated cells utilize the genetic information it does not *per se* explain differentiation. As often as not, differential gene activity may be a result of differentiation, rather than its causal mechanism. Moreover, another difficulty is attendant on the view that differentiation is best explained in terms of differential gene activity. It is that in the eukaryotic cell the activity of specific genes can rarely be monitored with any accuracy. This is because transcription of a gene into RNA is not necessarily followed directly by its translation into protein. Indeed, in some cases genes may be transcribed and the RNA never translated. Until such times as different messenger RNA molecules can be accurately partitioned and identified, we must depend on recognition of the specific protein as an indicator of the activity of

the individual gene. It should therefore be recognized that present ideas about differential gene activity during cell differentiation, monitored at the level of the protein product, are based largely on inference. Differential gene activity should be therefore seen as an important mechanism in the differentiation process, but not necessarily the primary causal one. How, after all, does a cell 'know' which genes should be expressed and which not?

This leads us to another point which needs to be stressed in relation to cell differentiation. It is that, even in the most complicated eukaryotic organism, there is a very limited number of different cell types. Very often great stress is put on the variety and number of cell types within a multicellular organism. But this should not blind us to what is perhaps even more impressive, namely that in an organism of many billions of cells there is likely to be only scores, or at the most a few hundred *different* cell types. Differentiation is a strictly limited exercise. Within any one cell type there may be many millions of essentially identical cells. Appreciation of this point of eukaryotic organization is surely fundamental to a proper understanding of cell differentiation. It tells us that, whatever mechanisms are involved, they are in many ways analogous to programme selection in a washing machine. The basic differing cell types may each be selected by a fairly simple switch, perhaps a single 'tissue specific master gene'. Once such a switch has been thrown, it will automatically select the appropriate programme of gene expression, turning on and off at appropriate times the many different genes relevant to that cell type. Such a programme selection must often be accomplished long before the cellular characteristics which it determines become overt, and it can clearly persist through many rounds of cell division. Once selected, the programme is normally remarkably stable and confusion with any other programme of gene expression very rare.

Let me stress these two points again because they are, I believe, fundamental to an enlightened view of differentiation. Firstly, differential gene expression, though no doubt important in cell differentiation, does not, of itself, explain how the initial commitment is made. Secondly, this initial commitment need only involve a choice between perhaps a hundred or so separate programmes of gene expression. The striking similarity of different cells of the same differentiated type probably reflects the identical nature of the programme selected within them.

In this book I have endeavoured to give a comprehensive view of the many interacting parameters which combine to induce and maintain cellular differentiation. The relative importance of these different aspects varies from one cell type to another and from one organism to another.

Since one of the easier ways of understanding what differentiation implies is seeing clearly what it does *not* imply, I have chosen to lead

in to the problem by way of chapter 1, which is partly devoted to a consideration of cells such as bacteria which are not differentiated from one another, and a look at the earliest symptoms of differentiation in primitive cells and organisms.

1

The evolutionary significance of differentiation

1.1 The origins of differentiation

Differentiation is normally taken to mean the process by which cells and tissues of multicellular organisms become different from one another. Such differences are no doubt one of the necessary consequences of multicellularity, since any organism with thousands or millions of cells is faced with problems of circulation, skeletal support, and movement. None of these difficulties can be overcome without some specialization in the form and function of different parts of the total cell mass.

Even in prokaryotic organization it is possible to recognize some anticipation of the process of eukaryotic differentiation. Thus, the switching on and off of bacterial operons (see p. 70) constitutes an altered commitment on the part of the cell and its metabolic machinery in response to the changing environment, while both division and sporulation demand an even closer commitment to a particular pathway. Some bacteria and blue-green algae form multicellular chains or aggregates, but little or no sign of cellular differentiation is to be found in such colonies. However, one remarkable group of prokaryotes which provides an example of true differentiation is the Myxobacteria. These are small rod-shaped unicellular organisms which form flat spreading colonies on solid media. One subgroup of the Myxobacteria, the fruiting Myxobacteria, will, under appropriate conditions, produce rather tight cell aggregates, within which differentiation occurs to produce large fruiting bodies (Dworkin, 1973). These form as brightly coloured shining droplets which rise above the surface of the cellular mass, and each consists of many spherical cells known as microcytes, a differentiated development from the normal rod shaped cell (Fig. 1.1).

Not only do the myxobacteria provide an example of true differentiation in prokaryotes, but they also form an outstanding example of evolutionary convergence, since the cellular slime moulds or *Acrasieae* engage in a similar process of aggregation and fruiting body production (see p. 7).

Although bacterial differentiation has been cited as an anticipation of eukaryotic differentiation, it seems unlikely that these prokaryotes form part of the main line of biological evolution. An organism like

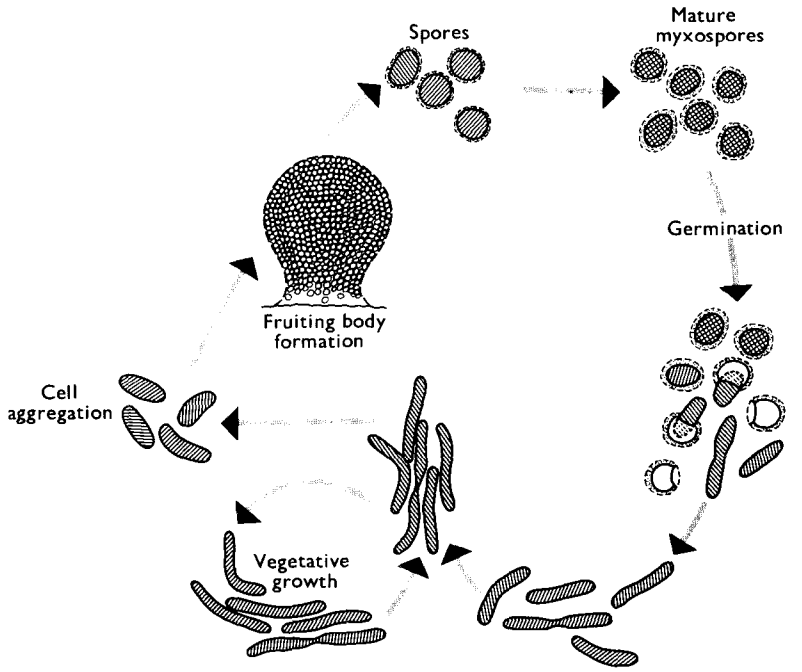

Fig. 1.1 The life cycle of *Myxococcus xanthus*, a member of the Myxobacteria. This figure should be compared to Fig. 1.7, which shows the life cycle of the unrelated organism *Dictyostelium*. (After Fig. 1, from Ashworth, J. M. and Smith, J. E., 1973, Microbial Differentiation. In *Symp. Soc. Gen. Microbiol.*, **13**. Cambridge University Press).

mycoplasma is perhaps a more likely common ancestor of both prokaryotes and eukaryotes. It is then of greater interest to consider the possibility that differentiation within unicellular eukaryotic cells may provide a more accurate appraisal of the origins of the complex pattern of differentiation found in eukaryotes.

For example, three important aspects of differentiation are demonstrated by the Protozoa. The first is a high degree of differentiation and specialization of parts of the single cell. Thus not only do we find mitochondria, chloroplasts, and cortex all possessing considerable independence from nuclear control but, in addition, particular parts of the cortex may be specialized to form sophisticated structures. These include the cilia and flagella which facilitate movement and the oral funnel which serves to channel food into the digestive vacuoles. Some of these features are well illustrated by the large flagellate *Trichonympha*, a symbiotic inhabitant of the gut of termites (Fig. 1.2). We should also notice that this compartmentalization of the Protozoan (and all other

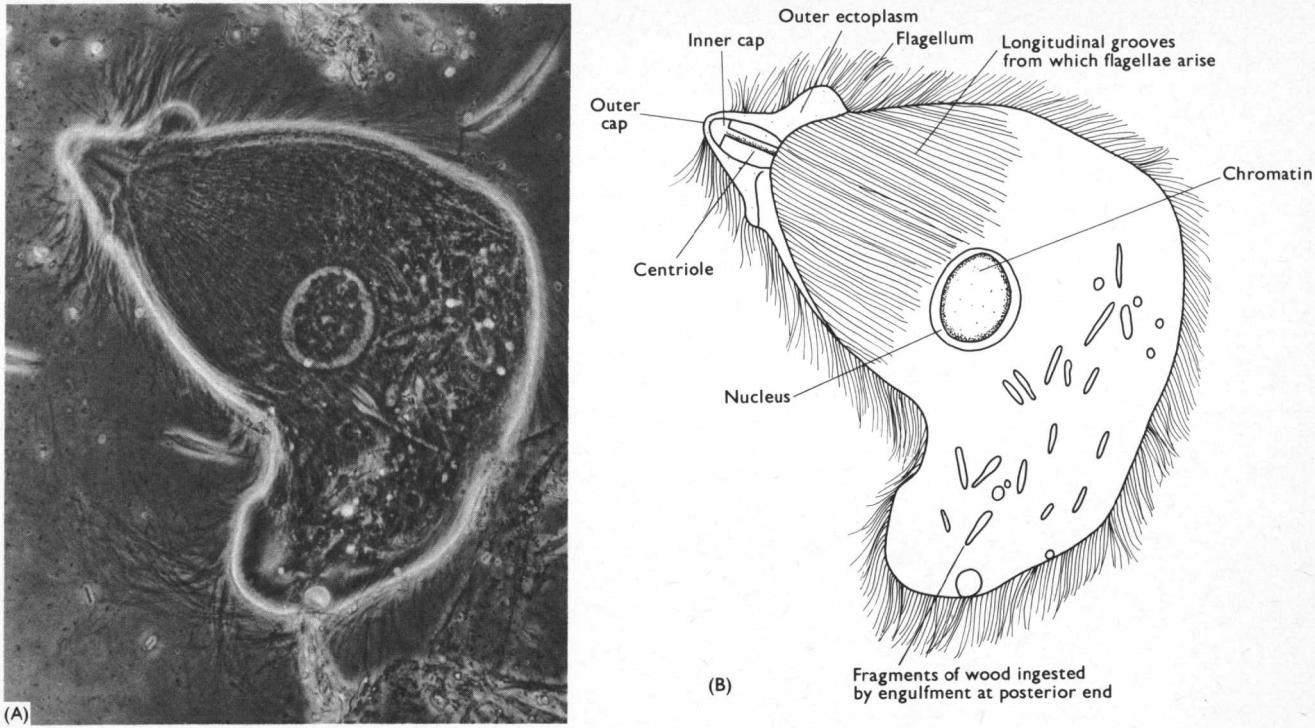

Fig. 1.2 (A) Phase contrast photograph of *Trichonympha campanulata*, from the gut of a termite. ×500 approx. (B) Diagram of the photograph shown in 1.2A.

eukaryotic cells) permits mutually incompatible reactions to proceed satisfactorily in different parts of the same cell.

The second aspect of differentiation found amongst Protozoa is the development of syncytial structure, that is the presence of many nuclei in one large acellular cytoplasmic mass. Within unicellular forms a variety of nuclear configurations may be found. Some, like the ciliates *Stentor* (Fig. 1.3) and *Microstomum*, have a highly complex polyploid macronucleus. Others like the large ciliate *Opalina* (Fig. 1.4), found as a common occupant of the frog rectum, have numerous small nuclei. Probably the most impressive multinucleate development in a single celled organism occurs in the life cycle of one group of Myxomycetes known as *Physarum*. The Myxomycetes or slime moulds are eukaryotic organisms related to the fungi. *Physarum* exists as separate amoeboid cells for part of its life history, but many of these amoebae may fuse to form a multinucleate plasmodium containing many thousands of nuclei (Dee, 1962; Sauer, 1973). These nuclei display a high degree of synchrony during DNA synthesis and division (Cummins, 1969). If food becomes scarce, cellular divisions appear within the plasmodium and differentiation into haploid spores takes place. Here we see an experiment in acellular organization, perhaps as an aid to synchronous division. Other syncytial organizations to be found in lower eukaryotes include those of the alga *Vaucheria* and many fungi.

The third aspect of differentiation displayed by Protozoa which merits attention is that of multicellular organization. Examples of this type of organization are rather few, the best occurring in the algae *Volvox* (Fig. 1.5) and *Hydrodictyon*. A fascinating series of algal species show increasingly elaborate multicellular organization, from the basic unicellular *Chlamydomonas* (Fig. 1.6) through *Pandorina* with 16 cells to *Volvox* (Fig. 1.5) with many hundreds. *Hydrodictyon* also displays a large three dimensional structure of very many cells but, like *Volvox*, there is little indication of a division of labour between the cells. Once again, it is amongst the slime moulds that the most interesting example is found. Some slime moulds are grouped under the name of *Acrasiae*. The best known member of this group is *Dictyostelium discoideum*, which, because it displays many characteristics which resemble true differentiation, is widely used as a research material.

Like *Physarum*, *Dictyostelium* normally exists as separate, free living amoeboid cells which wander over the soil surface, feeding on bacteria. When food is scarce, the behaviour of these amoebae changes dramatically. Instead of behaving indifferently to one another, the cells proceed to aggregate into a large tissue mass consisting of thousands of cells. This aggregate appears to result from the release of an attractive compound which has been shown to be cyclic AMP (see p. 138). The aggregate is known as a slug or grex and is capable of co-ordinated movement along light or temperature gradients. It is surrounded by a coating or sheath of slime but the mechanics of movement of the slug

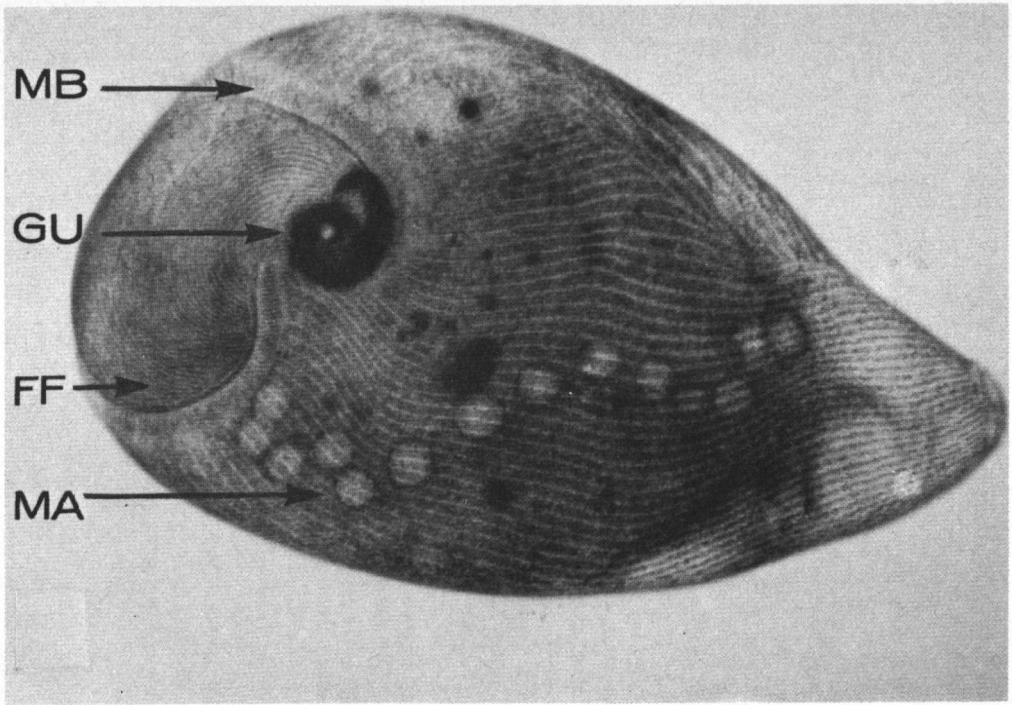

Fig. 1.3 Living *Stentor coeruleus* photographed in a micro-compression chamber. × 190. FF = frontal field; GU = gullet; MB = membranellar band; MA = macronuclear nodes. Numerous food vacuoles are to be seen in the cytoplasm. (After De Terra, N., 1970. *Symp. Soc. Exp. Biol.*, **24**, Copyright Academic Press Inc.)

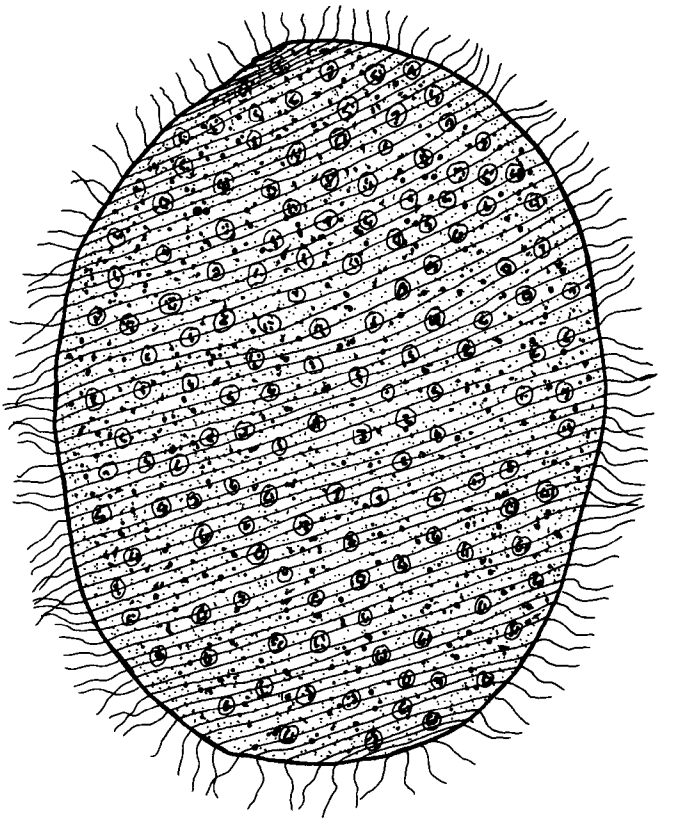

Fig. 1.4 Cell of *Opalina ranarum*, showing numerous nuclei. × 150 approx.

are not understood. Yet a further elaboration on this multicellular organization occurs if food remains scarce. In these circumstances the slug will stop moving, round up, and change into a fruiting body containing many spores (See Fig. 1.7). A remarkable differentiation occurs at this stage, since the stalk cells are those which travelled at the front of the slug, while those cells situated at the rear become spore cells (Ashworth, 1971) (See Fig. 1.8). The striking convergent evolution displayed by this phenomenon and spore production in the Myxobacteria has already been pointed out. In *Dictyostelium* we see perhaps the most impressive exploitation of differential organization shown by any single celled organism. It is also advantageous, as pointed out by

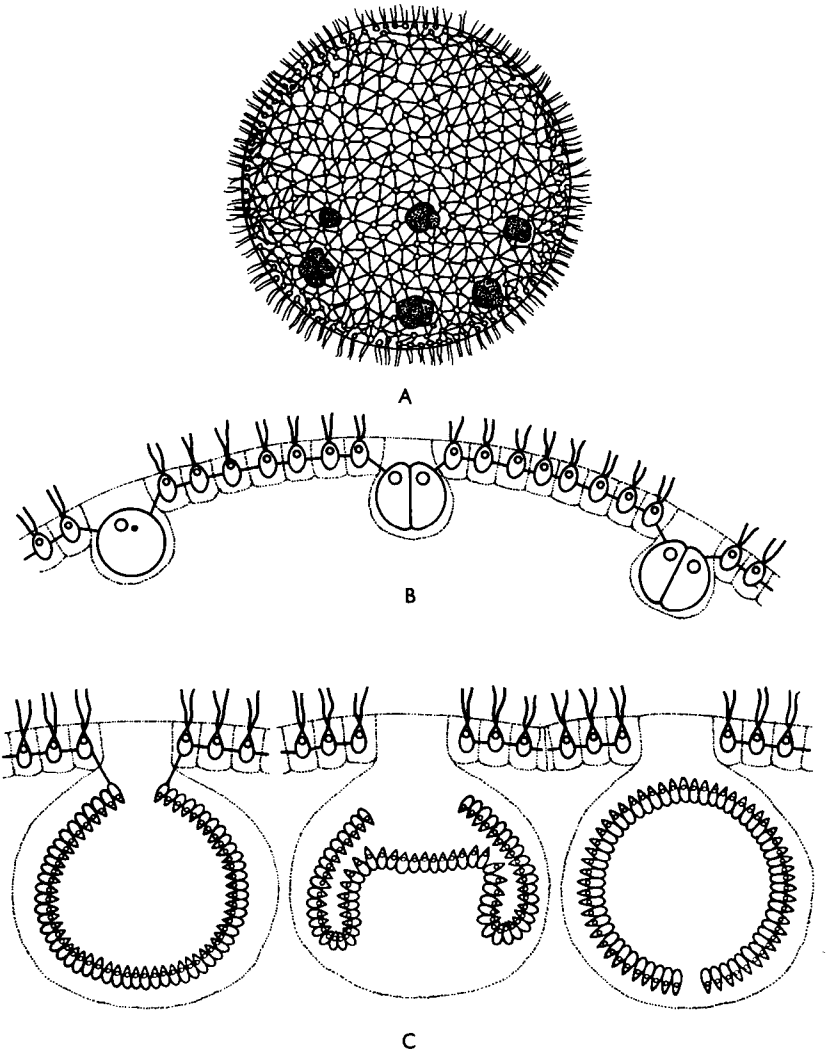

Fig. 1.5 (A) Colony of *Volvox*, with daughter colonies in the lower part of the sphere. (B) Enlarged view of part of a *Volvox* colony showing the cytoplasmic connections between adjacent cells and three daughter colonies commencing growth. (C) A similar section to B above, but showing inversion of the daughter colonies after formation of the definitive cell number. (A and B after Hyman, L., 1940. *The Invertebrates, Protozoa Through Ctenophora*. McGraw-Hill; C after Smith, G. M., 1955. *Crytogamic Botany*, Vol. 1 2nd edn. McGraw-Hill.)