

# Primordial Germ Cells in the Invertebrates

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# PRIMORDIAL GERM CELLS IN THE INVERTEBRATES

*From epigenesis to preformation*

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## Preface

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In his book *L'Origine des Cellules Reproductrices et le Problème de la Lignée Germinale* Bounoure (1939) summarised the knowledge of the origin of the germ cells in the animal kingdom which was available at the time. Much work has been done in the succeeding four decades, particularly on the ultrastructure of the germ cells in the insects and the anuran amphibians. The request of the editors of the Developmental and Cell Biology Series of Cambridge University Press to write a new, up-to-date monograph on the origin of germ cells in the animal kingdom therefore seemed to us well justified. However, we did not feel it right to treat the very extensive subject in a single volume. As we mentioned in the companion volume to this *Primordial Germ Cells in the Chordates* (Nieuwkoop & Sutasurya, 1979), we feared that a single volume would not only be difficult to write, but also hard to read.

Our personal contributions to the subject have dealt with the origin of the germ cells in the vertebrates, in particular in the urodele amphibians, where the germ cells show both a site and a mode of origin that are essentially different from those in the anuran amphibians. This discrepancy, in our opinion, points towards a different phylogenetic origin of the two groups of Amphibia. The chordates, of which the vertebrates are the main representatives, form a rather homogeneous group, the phylogeny of which has been widely studied due to the availability of an extensive fossil record. In the companion volume we therefore treated the origin of the germ cells in the chordates against the background of early embryogenesis as well as phylogeny.

Unfortunately, in the invertebrates, the origin of the germ cells cannot be treated in the same way, since too little is known about the phylogenetic relationships among the various invertebrate phyla. Germ cell origin in the invertebrates must therefore, of necessity, be discussed against the background of their embryonic development only. However, in most instances, this knowledge is of a purely descriptive nature. Fortunately, there are a number of groups where experimental analysis has given us some insight into the mode of origin of the germ cells. Emphasis will be placed on these groups.

We will discuss primarily how far the origin and development of the germ

cells in the various invertebrate groups can be characterised as *epigenetic*, and how far as *preformistic* (for definitions of these concepts see Hertwig, 1900 (reprinted in 1977), Raven, 1958 and Maresquelle, 1978). In the introduction we try to formulate some basic questions concerning different mechanisms which may act in germ cell formation. After discussing germ cell formation in the various phyla, we will try to formulate some common principles of germ cell development in the concluding chapter. We hope that such a treatment will stimulate interest as well as lead to active participation of developmental biologists in this fascinating field of research.

## Acknowledgements

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First of all we would like to thank the editors of the Developmental and Cell Biology Series of Cambridge University Press for allowing us to treat the subject of the origin of the germ cells in the animal kingdom in two separate volumes, the first on the chordates and the second on the invertebrates.

We are very grateful to the Department of Biology of the Institut Teknologi Bandung for granting leave to L.A.S. in order to come to Holland for the extensive literature study for the second book and the final preparation of the manuscript, and for the hospitality extended to P.D.N. during the preparation of the definitive text.

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# 1

## General introduction

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Towards the end of the nineteenth century Weismann formulated his theory of the functioning of heredity in development, laid down in his classical works *Die Continuität des Keimplasmas als Grundlage einer Theorie der Vererbung* (1885) and *Das Keimplasma. Eine Theorie der Vererbung* (1892). According to this theory pluricellular organisms consist of two main components, the somatic cells, constituting the body of the individual of a particular generation, and the germ cells, representing the forerunners of the next generation. The two components were called *soma* and *germen* respectively.

The analysis of the origin of the germ cells has been strongly influenced by the theoretical concepts of *soma* and *germen*. Weismann postulated that the development of the unicellular egg into a complex organism with different cells, tissues and organs was based upon the differential distribution of the various *Determinanten* (later called genes) amongst the different cell types, allowing each cell type a specific but restricted mode of development. The only exception to this rule would be the germ cells, which would retain the full complement of *Determinanten* originally present in the fertilised egg, thus forming the so-called 'germ line'.

The distinction between *soma* and *germen* as formulated above is necessarily an essential and permanent one, since the somatic cells, which have received only part of the genetic complement of the egg, would no longer be able to form *totipotent* germ cells. Conversely, the germ cells would at any time be able to form somatic cells of the organism.

In the years that followed many investigators have shown that, in both the vertebrates and the invertebrates, the germ cells either segregate from the somatic cells very early in embryonic development, or are discernible only at much later stages of development. In the latter situation, during a rather long initial period of development, no clear distinction between *germen* and *soma* can be made. Bounoure (1939) reviewed the literature up to the late 1930s on the early versus late appearance of the germ cells in the various groups of the animal kingdom in his book *L'Origine des Cellules Reproductrices et le Problème de la Lignée Germinale*. Referring primarily to the vertebrates, Cambar (1956) called the early segregation of the germ cells the *preformistic* mode of germ cell formation; this is often characterised by the presence of a special cytoplasmic structure, the 'germ plasm', which acts as a

## 2 General introduction

germ cell determinant. He called the late appearance of the germ cells the *epigenetic* mode of germ cell formation; this often seems to occur under inductive influences. The first question to be answered is whether this distinction also holds for the invertebrates, and if so, whether it has the same or a broader significance there.

What is our present insight into the potentialities of germ cells and somatic cells? We know that during the subdivision of the egg into a large number of cells forming different cell types, the division of the nucleus is *not* characterised by a differential distribution of genes among the daughter nuclei, but that the accurate replication of the full complement of nuclear genes and the subsequent distribution of the two identical sets of genes among the daughter nuclei renders them potentially isopotent. This has been demonstrated convincingly by, among other methods, transplantation of nuclei from differentiated somatic cells into enucleated eggs (see reviews by Gurdon & Woodland, 1968 and Gurdon, 1974*a* on vertebrates, and by Gurdon, 1974*b* and Illmensee, 1976 on insects). In addition, it has been shown that different genes are active in different phases of development and in different cell types (see review by W. Beermann, 1967). This has led to the conclusion that the differentiation of cells is due to differential gene activation or derepression and not to differential distribution of genes, so that Weismann's theory has been essentially refuted. We must therefore ask ourselves whether the distinction between *soma* and *germen*, which is a direct consequence of Weismann's theory, should not be abandoned as being inadequate and obsolete. Since we will argue that it should, the 'germ line' concept will be avoided as much as possible in the following chapters.

On the basis of the above considerations Davidson (1976) has advanced the hypothesis that in the nuclei of different cells different genes are repressed or derepressed due to the different cytoplasmic composition of the cells. This hypothesis, for which Davidson and others brought together extensive evidence, places the distinction between *soma* and *germen* in an entirely different light. Germ cell and somatic cell nuclei are *potentially* equal, since they both contain the full complement of genetic information. Due to their different cytoplasmic composition, however, germ cells and somatic cells may express different potentialities due to the activation of different parts of the genome.

An interesting question arises here. Are the more restricted expressions of the somatic cells permanent or only temporary? In other words, can the differential inactivation of genes in somatic cell nuclei be reversed under certain conditions? Transplantation of nuclei from differentiated somatic cells into enucleated eggs has demonstrated that, in a low percentage of cases, such nuclei *can* support normal development, so that somatic cell nuclei *can* reacquire their full potentialities. However, transplanted germ cell nuclei do so in an appreciably higher percentage of cases. This relative

difference is probably due to the fact that germ cell nuclei can adapt more easily to the special requirements of the egg cytoplasm than can the nuclei of differentiated somatic cells.

Germ cells are considered to be *totipotent* since they can give rise to complete new individuals. The question should, however, be raised of whether totipotency is actually an adequate concept. Embryonic development, including germ cell formation, requires at any time a very accurately programmed, sequential release of the potentially present, but functionally repressed, genetic information. Moreover, it should be realised that, although the nuclei of egg and sperm may be more or less equivalent – in androgenesis the sperm nucleus can support normal development of the enucleated egg almost equally as well as the female pronucleus of a parthenogenetically activated egg – they belong to entirely different, specialised cell types. They must therefore have different sets of derepressed genes. One must conclude that the concept of totipotency of the *germen* is inadequate, and that the distinction between *germen* and *soma* on the basis of fundamental differences in potentialities is erroneous. The distinction between *germen* and *soma* is only a relative one. This makes it theoretically possible that somatic cells can be converted into germ cells.

According to Davidson's hypothesis, the cell-type-specific machinery keeps the somatic cell nucleus engaged in releasing only that genetic information which is relevant for a particular differentiated state. Conversion of a somatic cell into a germ cell is possible, therefore, only after the specific cytoplasmic differentiation of the somatic cell has been 'erased'. In other words, the somatic cell must have dedifferentiated to such an extent that the information for other types of differentiation can be released; in its most extreme form this is the information for germ cell formation. Consequently, the more highly differentiated a somatic cell is, the more unlikely it is that it can be converted into a germ cell.

A clear distinction must be made between nuclear potentialities and cellular expression. Although nuclei of differentiated somatic cells *can* support normal development of enucleated eggs, in normal development somatic cells are rarely transformed into germ cells. This holds particularly for the more highly evolved forms, such as holometabolous insects and vertebrates. These are generally characterised by a relatively early segregation of the germ cells during embryonic development, as well as by the non-replaceability of the germ cells. On the other hand, the non-convertibility of *soma* into *germen* and vice versa certainly does not hold for several lower animal phyla, such as the sponges and the coelenterates. As well as a sexual form of reproduction with gamete formation, the majority of these animals show an asexual form of reproduction by bud formation or schizogenesis. The two forms of reproduction often alternate.

The germ cells of highly evolved forms, which are usually segregated

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during early embryonic development, are often characterised by the presence of a special cytoplasmic structure which is supposed to act as a germ cell determinant (see Hegner, 1914 and Gehring, 1976b), giving germ cell development in these forms a strongly *determinate* or *preformistic* character. This aspect has been studied extensively in several holometabolous insects as well as in the anuran amphibians. How general is this phenomenon among the invertebrates? Do the special organelles encountered in the germ plasm actually act as a germ cell determinant? In our previous book, dealing with primordial germ cells in the chordates (Nieuwkoop & Sutasurya, 1979), this notion has been seriously questioned. What is the evidence for it in the invertebrates?

Weismann's germ plasm theory has been rejected on the grounds of the essential equipotentiality of all the nuclei in a developing organism. What, then, is the significance in this context of the phenomena of chromosome elimination and chromatin diminution encountered in the somatic cells of certain nematodes, crustaceans and insects? Do these phenomena support Weismann's theory? Is it really a unique part of the genetic information that is eliminated, or only reduplicated chromosomes or amplified genes?

Contrary to the highly evolved forms, where germ cell development seems to be strongly preformistic, many lower invertebrate forms show a more or less typical *epigenetic* mode, where the germ cells are formed from cells of one or the other 'germ layer' under inductive influences from adjacent organ anlagen, or even under the influence of external environmental factors. Among the vertebrates a typically epigenetic mode of germ cell development has been demonstrated in the urodele amphibians. Is this also encountered in invertebrate groups? Is the mechanism in invertebrates and vertebrates the same or different? Can a more-or-less continuous transition between the typically epigenetic and the typically preformistic modes be found among the invertebrates, or are the two modes of germ cell formation mutually exclusive?

Whatever the answer to these questions, the essential problem with which we are faced is whether we can find a denominator common to the different modes of germ cell formation, the end-product being the same in all cases. The solution to this problem may lead to a deeper understanding of germ cell formation generally. We must ask ourselves whether indications can be found that germ cells show a particular form of activity or inactivity of the genome which is conditioned by a specific composition or functioning of their cytoplasm. If we cannot yet define such requirements, how should we proceed towards this goal?

We now come to some more practical points which have to be settled before we can start the survey of the various groups of invertebrates.

As mentioned in the Preface, we know hardly anything about the phy-

logenetic relationships among the different invertebrate phyla. Consequently, the invertebrates can be arranged only according to a purely *taxonomic* classification. It is evident that classification into separate phyla and their arrangement in a hierarchical order from 'lower' to 'higher' forms of organisation is subjective. Opinions differ rather strongly among the taxonomists themselves; consequently, several schemes have been proposed. We have, on the whole, followed the classification of the Dutch Leyden School, which is based mainly on the work of Hyman (1940, 1951*a,b*, 1955, 1959, 1967), Barnes (1963) and Karstner (1965/7). The primary subdivision of the Metazoa into Radiata and (acoelomate, pseudo-coelomate and eucoelomate) Bilateralia makes for easy surveyability. It should, however, be regarded as a purely practical one. The classification used in this book is given in table 1.1. The well-known phyla and classes will be treated in this sequential order. However, there are a number of metazoan phyla in which the mode of germ cell formation is almost completely unknown. These will be discussed briefly in a separate chapter at the end of the systematic part.

Table 1.1. *Classification of the animal kingdom used by the authors based mainly on Hyman (1940, 1951*a,b*, 1955, 1959, 1967), Barnes (1963) and Karstner (1965)*

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Kingdom ANIMALIA	
Subkingdom Protista	
Phylum Protista	
Subkingdom Parazoa	
Phylum Porifera	
Subkingdom Metazoa	
Division Radiata	
Phylum Coelenterata or Cnidaria	
Phylum Ctenophora	
Division Bilateralia	
Subdivision Acoelomata	
Phylum Platyhelminthes	{ Class Turbellaria Class Trematoda Class Cestodes
Phylum Nemertini	
Phylum Mesozoa	
Subdivision Pseudocoelomata	
Phylum Acanthocephala	
Phylum Rotifera	
Phylum Gastrotricha	
Phylum Kinorhyncha	
Phylum Nematomorpha	
Phylum Nematoda	
Phylum Entoprocta	

Table 1.1 continued

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Subdivision Eucoelomata							
Phylum Brachiopoda							
Phylum Ectoprocta							
Phylum Phoronida							
Phylum Annelida							
Phylum Echiurida							
Phylum Sipunculida							
Phylum Priapulida							
Phylum Echinodermata							
Phylum Mollusca							
Phylum Tardigrada							
Phylum Pentastomida							
Phylum Onychophora							
Phylum Arthropoda	<table><tr><td>Class Myriapoda</td><td rowspan="4">{</td><td rowspan="4">Apterygota Pterygota</td></tr><tr><td>Class Hexapoda</td></tr><tr><td>Class Crustacea</td></tr><tr><td>Class Chelicerata</td></tr></table>	Class Myriapoda	{	Apterygota Pterygota	Class Hexapoda	Class Crustacea	Class Chelicerata
Class Myriapoda	{	Apterygota Pterygota					
Class Hexapoda							
Class Crustacea							
Class Chelicerata							
Phylum Chaetognatha							
Phylum Pogonophora							
Phylum Hemichordata							
Phylum Chordata							

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In each of the various groups germ cell formation will be classified (often tentatively) into one of the following three main modes: the typically *epigenetic* mode, an *intermediate* mode with relatively late appearance of the germ cells, and the typically *preformistic* mode. In the concluding chapter we shall try to arrange the various phyla and classes into a more-or-less continuous series. We realise that such an arrangement is, again, a subjective one. We hope, however, that it will make the discussion more adequate because, on the one hand, it emphasises the great diversity in modes of germ cell formation, even in related groups, while, on the other hand, it points towards the possible existence of a common mechanism of germ cell formation.

For each of the phyla or classes, early embryonic development (as well as asexual reproduction and regeneration) will be discussed first, wherever appropriate, and will be followed by a discussion of germ cell origin serving to place germ cell formation against the background of embryonic development.

It would be desirable to view germ cell formation against the background of both descriptive and experimental analysis of early development, so that not only the *site* but also the actual *mode* of germ cell formation could be elucidated. It must, however, be realised that in many groups hardly any



experimental analysis has been performed, so that, in these cases, nothing definite can be said about the actual mode of germ cell formation. In other groups, even normal development is only fragmentarily known, and germ cells are recognised at only rather late stages of development, making even their site of origin questionable. Groups of which we know so little must necessarily be treated briefly. In the more thoroughly studied groups, normal development is discussed in broad outline only, since this is not a textbook of invertebrate development. A similar restriction holds for the references on normal development, since in certain groups, such as the insects, the literature on normal development is far too extensive for complete coverage. We shall, therefore, refer mainly to review articles, citing the original publications only for aspects of particular interest. For further information the reader will find the full titles in the reference lists of the reviews cited here.

As in our previous book (Nieuwkoop & Sutasurya, 1979), we shall restrict ourselves to the initial development of the germ cells, leaving oogenesis and spermatogenesis out of consideration. This means that the book will deal only with the *primordial germ cells* (PGCs). We shall treat the subject as comprehensively as possible, but in a concise form which we hope will ensure easy reading. Given the excellent review of the older literature by Bounoure (1939), we shall restrict ourselves mainly to the literature of the last four decades, referring to older literature only where no recent literature is available or where reference to important older investigations cannot be omitted.

Some of the relevant terminology must be discussed briefly here. The development of the germ cells from their first detectable origin until their release as mature gametes, called gametogenesis, is subdivided into two major periods, that preceding and that succeeding sexual differentiation. During the first period the germ cells are called 'primordial germ cells' (PGCs). The forerunners of the germ cells, while they are segregating from the somatic cells of the embryo, will be called 'presumptive primordial germ cells' (pPGCs), in contradistinction to the fully segregated or 'true' primordial germ cells. For more detailed information the reader is referred to the chapter on terminology in the companion volume, *Primordial Germ Cells in the Chordates* (Nieuwkoop & Sutasurya, 1979, pp. 5–7).

In the invertebrates the gonads may either be formed from cells split off from the oogonia and spermatogonia, which come to surround the germ cells, or may develop independently of the PGCs, often in another part of the embryo. In the latter case the PGCs must move actively or passively from their site of origin to the 'gonadal anlagen'. The latter may differentiate into an 'ovary', a 'testis' or an 'ovotestis'. In some forms the sexes are separate, and male and female animals can be distinguished. In so-called