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Primordial germ cell development: is the urodele pattern closer to mammals than to anurans?

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ABSTRACT All animals can be classified into three types depending on their modes of germ cell formation; epigenetic, intermediate and preformistic. In urodeles, which show the intermediate mode, primordial germ cells (PGCs) are morphologically recognized at first in early tailbud embryos. The PGCs, which are located within the lateral plate mesoderm, are induced as part of the regional induction of the mesoderm by the vegetal yolk endoderm. No cytologically distinctive, germ cell-specific germ plasm can be detected during early development of urodeles. *'Nuage'* materials, which are specific to germ line cells in almost all animals, do, however, appear in the cytoplasm of the urodele PGCs during later embryogenesis. In contrast, PGCs in anurans are preformistically established under the influence of germ plasm. Because all germ cells, once established, show virtually identical behavior, regardless of whether different modes of germ cell formation are employed, the basic mechanism of germ cell formation and differentiation in all animals could be similar at the molecular levels. Although the molecules involved in germ cell formation in amphibians have not been identified, many aspects of germ plasm formation in anurans are similar to *Drosophila*, in which three classes of genes involved in germ cell formation have been identified: Class I genes are necessary for pattern specification during germ cell formation, Class II for the assembly of germ plasm components, and Class III for germ cell segregation. Assuming that germ cell formation in all animals requires the expression of all such genes, the three modes of germ cell formation mentioned above could be explained in terms of spatio-temporal expression of genes which are similar to those that have been identified in *Drosophila*. A tentative model of gene regulation for the three different modes of germ cell formation has been proposed in terms of temporal expression of these three classes of genes.

KEY WORDS: *PGCs, urodele, epigenesis, preformation*

Introduction

Most of the cells which constitute the body of multicellular organisms (i.e., somatic cells) inevitably die after a certain number of cell divisions. In contrast, germ cells, which differentiate to gametes and are responsible for the continuity of a species, are potentially immortal (see Wakahara, 1990a). Historically, three modes of germ cell formation in the animal kingdom have been proposed (Nieuwkoop and Sutasurya, 1979); (1) epigenetic, (2) intermediate, and (3) preformistic (Fig. 1). They are summarized below:

1. In the *epigenetic* mode, sexual and asexual forms of reproduction alternate under the influence of environmental factors. During sexual reproduction germ cells are formed from undifferentiated or dedifferentiated embryonic cells (i.e., totipotent embryonic cells, TECs). During asexual reproduction, the TECs give rise to various somatic cell types (in the Cnidaria and Platyhelminthes).
2. In the *intermediate* mode, germ cells are formed at a rather late stage of development from pluripotent embryonic cells (PECs) which earlier must have passed through a phase of somatic development. Once formed, however, they are no longer replaceable by other cells (in mammals, urodeles and so on).
3. In the *preformistic* mode, germ cells segregate from somatic cells at a very early stage of embryonic development. They are often predetermined by the presence of a germ cell-specific germ plasm, so that either presumptive or true germ cells can be distinguished during most, if not all, of the life cycle (in insects, anurans and so on).

Irrespective of the different modes of germ cell formation, all germ cells, once established, show virtually identical behavior. They undergo meiosis, which is specific to germ cells, and differentiate into female and male gametes, regardless of which mode of germ cell formation was employed. Furthermore, it seems reasonable to assume that the molecular mechanism which controls fundamental phenomena, such as conversion of cell division from mitosis to meiosis, pairing of homologous chromosomes during meiotic prophase, and the bisexual differentiation of germ

Abbreviations used in this paper: PGCs, primordial germ cells.

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cells, is basically the same in all animals. Such conservation of basic molecular mechanisms in embryonic patterning is well known (reviewed by Scott, 1994).

This review briefly describes the normal development and possible origin of germ cells in the Urodela (intermediate mode) in comparison with the preformistic mode of germ cell formation employed by Anurans. In addition, this review speculates that the formation of germ cells in the Urodela and the Anura is regulated by basically the same mechanism, even though they display superficial differences in germ cell formation.

Morphological studies on the germ cell formation in the urodela

Characteristics of PGCs in Urodeles

Humphrey (1925) first reported that germ cells could be recognized in urodelan development as early as the tailbud stage by virtue of such characteristics as large spherical nuclei and finely dispersed chromatin. Such cells were located within the lateral plate mesoderm (Fig. 2A). Studies on germ cell formation in urodeles since Humphrey's initial report have appeared less frequently than in anurans and other chordates, because no proper markers for identification of presumptive PGCs in urodeles exist.

For instance, in anurans a special cytoplasmic structure, the so-called *germinal cytoplasm* or germ plasm, which is originally located in the subcortical layer of the vegetal region of the fertilized egg, and later in the PGCs of the tadpole, is used as a reliable cytoplasmic marker of presumptive PGCs and true PGCs (Blackler, 1958, 1966). In *Xenopus*, the germ plasm has been reported to be continuously present from ovarian oocytes to fertilized eggs (Czolowska, 1969). During the first and second cleavages the germ plasm is partitioned, more or less equally, between the first four blastomeres (Dixon, 1981; Akita and Wakahara, 1985). During subsequent cleavages, only one daughter cell of each pair receives the germ plasm, generating the founder clone of four germ plasm-containing cells or presumptive PGCs. During blastulation, the germ plasm moves from the peripheral cytoplasmic position it occupied during earlier stages to a position in contact with the nuclear membrane at the gastrula stage (Blackler, 1970). Thus, presumptive PGCs can be identified throughout early development by the presence of germ plasm in the cytoplasm (Blackler, 1958; Whitington and Dixon, 1975; Kamimura *et al.*, 1976) (Fig. 2B).

McKay *et al.* (1953) were the first to demonstrate higher alkaline phosphatase activity in the cytoplasmic rim of PGCs in mammals. This was confirmed by many investigators and used as a reliable marker for extragonadal PGCs in mammals (for review see Eddy, 1975; Nieuwkoop and Sutasurya, 1979). The alkaline phosphatase activity in mammalian PGCs can be detected at the electron microscopical level (Clark and Eddy, 1975).

In birds, some cytochemical features can be used to characterize the PGCs. The most prominent feature of chick PGCs is their high content of PAS-positive materials (glycogen). This was first demonstrated by McKay *et al.* (1953), and later confirmed by many authors (see Eddy, 1975; Nieuwkoop and Sutasurya, 1979). Large amount of PAS-positive material have also been reported in the extragonadal PGCs of reptiles (Milaire, 1957).

As described above, presumptive PGCs of urodeles have no specific markers for their identification, while many groups of other

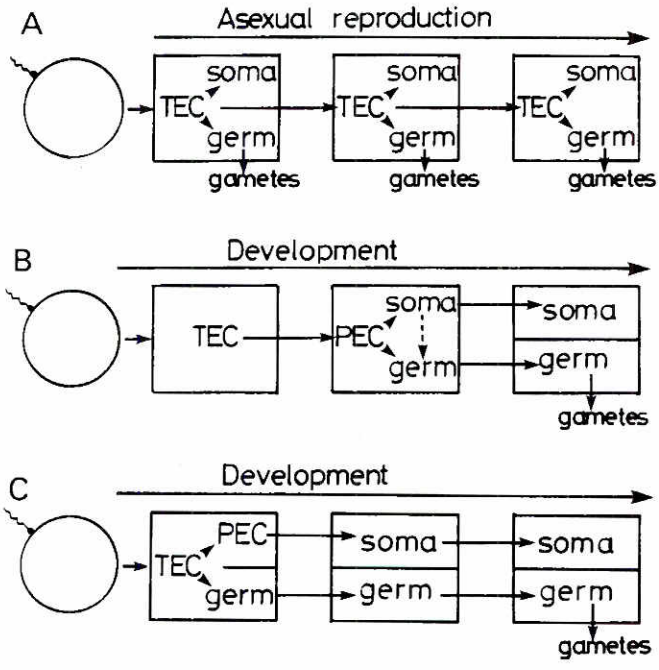

Fig. 1. Diagrammatic representations of germ-soma relationship in various animals, illustrating the three modes of germ cell formation; *epigenetic* **(A)** *intermediate* **(B)** *and preformistic* **(C). (A)** *In the epigenetic mode totipotent embryonic cells (TECs) give rise to all somatic cells during an asexual form of reproduction. Before asexual reproduction germ cells differentiate from the TECs under the influence of environmental factors.* **(B)** *In the intermediate mode germ cells are formed at a later stage of development from TECs and/or pluripotent embryonic cells (PECs).* **(C)** *In the preformistic mode germ cells segregate from somatic cells at a very early stage of embryonic development due to the presence of a germ cell-specific germ plasm.*

chordates show certain morphological or cytochemical features specific to the presumptive PGCs or extragonadal PGCs. This absence of a specific marker has led to difficulty in studying both the origin of the PGCs and the mode of formation of germ cells in urodelan embryos.

Ultrastructural studies on germinal granules and nuage

At the electron microscopical level, germ plasm in anuran eggs and embryos contains numerous mitochondria and small electron-dense bodies (so-called germinal granules) (see Eddy, 1975; Nieuwkoop and Sutasurya, 1979). These granules are composed of small electron-dense foci, which appear to be embedded in a matrix of extremely fine fibrils (Williams and Smith, 1971), and are believed to have a "germ cell-forming activity" (Wakahara, 1977, 1978; Ikenishi *et al.*, 1986). They are frequently found in contact with mitochondria. The origin of germinal granules in *Xenopus* has been studied by Heasman *et al.* (1984), who recognized germinal granules in the "mitochondrial cloud" of stage I oocytes. Ikenishi and Kotani (1975) have described ultrastructural changes of the germinal granules during *Xenopus* development. At early stages germinal granules in the presumptive PGCs show a fibrillogranular structure, but they soon change, first into irregular string-like bodies, and then, at the feeding tadpole stage, into granular material within

the PGCs, suggesting transformation into *nuage* (the French word for "cloud") materials.

Williams and Smith (1971) were able to observe electron-dense bodies in ultra-thin sections taken from the marginal zone of a fertilized axolotl egg. These structures were similar to anuran germinal granules. Unfortunately, however, this observation has not been confirmed by other investigators. Subsequent attempts to identify electron-dense structures of the type described above during cleavage and early embryogenesis have not been undertaken in urodeles, owing to the difficulties in localizing and identifying cytoplasmic regions (or cells) which might be expected to contain germinal granules (Smith *et al.*, 1983).

Specific structures corresponding to germinal granules or their derivatives in anuran embryos were first recognized in the PGCs of *Ambystoma mexicanum* at stage 40 (late tailbud) (Ikenishi and Nieuwkoop, 1978). Such structures (*nuage* materials), were not found in PGCs prior to stage 40. Between stages 40 and 46 (pre- to post-hatching), the amount of the *nuage* materials markedly increased.

Similar structures, not seen in somatic cells, were found in PGCs of feeding larvae of the newt, *Triturus* (presently termed *Cynops*) *pyrrhogaster* (Hamashima and Kotani, 1977). These structures resemble those of the *nuage* materials found in the oocytes of anurans. The *nuage*, an electron-dense cytoplasmic compartment specific to the germ line cell, was found to be closely associated with a metaphase chromosome in the spermatogenic cells of *Cynops pyrrhogaster* (Hamashima and Kotani, 1979). From those observations described above, it thus seems that *nuage* materials appear in urodelan PGCs during later embryogenesis.

Nuage materials have been recognized in the cytoplasm of germ cells in numerous animals from coelenterates to mammals (see Eddy, 1975; Nieuwkoop and Sutasurya, 1979, 1981). The universal presence of the *nuage* in differentiated germ cells, such as oogonia and oocytes, in many species suggests that the *nuage* is only an indication of germ cell differentiation rather than a causal factor for germ cell determination (see Wakahara, 1990b). The relatively late appearance of *nuage* materials in urodelan PGCs suggests that it is a morphological manifestation which develops as a result of germ cell determination, and that it will function in later phases of germ cell differentiation.

Experimental studies on the germ cell formation in the urodela

Origin of pgcs in urodelan embryos

The extragonadal origin of PGCs is well established in all vertebrates. In anurans, PGCs originate from the deep endoderm. The presumptive PGCs can be identified throughout early development by the presence of germ plasm, which was originally located in the subcortical layer of the vegetal region of eggs (Fig. 2B). In contrast to the apparent endodermal origin of anuran PGCs, the primary site of origin of PGCs in other chordates is not so well understood, mainly because it is at present difficult to trace the precursor cells of PGCs with conventional cytological techniques. Thus, the origin of PGCs needs to be analyzed by experimental techniques such as the classical transplantation and/or extirpation methods previously used for urodelan embryos (Humphrey, 1927), microdissection of early embryos in chick (Ginsburg, 1994), and clonal analysis of single epiblasts of early mouse embryos using cell lineage labels (Lawson and Hage, 1994).

Lawson and Hage (1994) have shown in mouse that PGC precursor cells are found in the proximal epiblast close to the extraembryonic ectoderm. That is, they are located in the presumptive extraembryonic mesoderm in both pregastrulation and early-streak stage embryos. These observations indicate that mouse PGCs are of extra-embryonic mesodermal origin.

Humphrey (1927, 1928, 1929) discovered the localization of PGCs in the medial to dorsal lateral plate mesoderm by unilateral extirpation and by transplantation of this portion of the mesoderm to the ventro-lateral side of host embryos at tailbud stages of *Ambystoma* embryos. Unilateral extirpation led to the complete absence of PGCs on the operated side, and transplantation led to additional germ cell formation. Similarly, Nieuwkoop (1947) reported that removal of the presumptive lateral plate mesoderm at the early neurula stage led to complete sterility or to a very marked reduction in the number of PGCs. These experiments demonstrated convincingly that in urodeles PGCs are of mesodermal

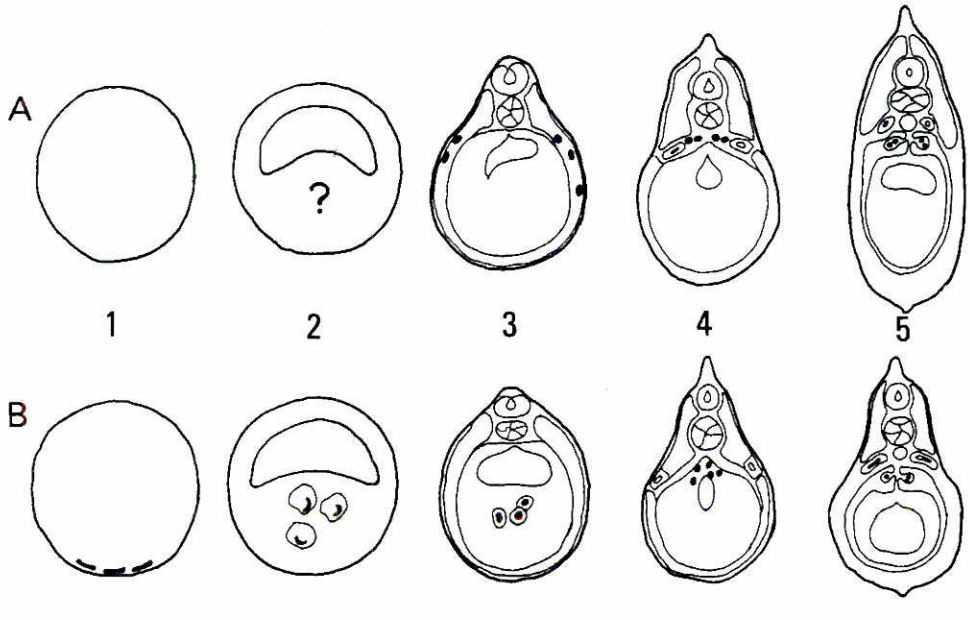

Fig. 2. Diagrammatic illustrations showing the origin, location and migration of germ cells in urodeles (A) and anurans (B). *1, fertilized egg; 2, blastula; 3, early tailbud; 4, late tailbud; 5, swimming larvae or tadpole. Because no distinct cytoplasmic markers such as the germ plasm of anurans are known, and since germ cells are formed after mesodermal induction, germ cells cannot be identified until the early tailbud stage in urodeles. In anurans presumptive PGCs and true PGCs can be identified throughout early development by the presence of germ plasm which originally locates in the subcortical layer of the vegetal region of eggs. Although the origin of germ cells is apparently different (i.e., mesodermal origin in urodeles and endodermal origin in anurans), they migrate to and eventually reach the germinal ridges at the swimming stage.*

origin and are distributed throughout the presumptive lateral plate mesoderm (Fig. 2A). Thus, it seems reasonable to conclude that the mesodermal origin of urodelan PGCs is basically identical to the mammalian pattern, but not to the endodermal origin of anuran PGCs.

Epigenetic formation of pgcs in urodeles

The idea that somatic cells can be induced to form PGCs was initially proposed by Kotani (1957, 1958). He removed presumptive lateral plate mesoderm from early gastrulae of *Triturus* (presently *Cynops*) *pyrrhogaster* and replaced it with presumptive ectoderm from other early gastrulae. In a majority of the operated embryos (examined at larval stages) PGCs, albeit reduced in number, were observed in the genital ridges. Kotani concluded from these studies that these PGCs were regulatively formed from the grafted ectoderm.

This conclusion is apparently supported by the experiments of Sutasurya and Nieuwkoop (1974). They made xenoplastic recombinants of animal ectodermal caps with the ventral endoderm of urodele blastulae. The animal caps of blastulae were divided into central, intermediate, dorsal, ventral and lateral pieces and recombined with half of the ventral yolk mass. The presence of PGCs was recognized in all combinations, but a dorsal-ventral as well as centro-lateral gradient was observed with respect to germ cell numbers.

The question of the origin of PGCs in urodeles was again approached in an extensive series of deletion and recombination experiments (Michael, 1984). Parts of blastula presumptive ectoderm from urodeles (*Ambystoma mexicanum*, *Triturus alpestris*) were combined with anuran (*Xenopus laevis*, *Bufo orientalis*, *Discoglossus pictus*) yolk masses of the same age. Nieuwkoop's (1969) demarcation of urodele blastula into four zones (Fig. 3) was used as a guide in preparing the various parts for recombination. When urodelan zone I ectoderm was combined with urodelan zone IV endoderm, a small number of recombinants contained the PGCs. In all combinations of urodele zone I ectoderm with anuran zone IV endoderm, the anuran cells dropped off from the recombinants within a day. Nevertheless, the remaining urodelan ectodermal tissues continued to differentiate, resulting in the formation of several mesodermal derivatives including PGCs. Thus, it is concluded that PGCs can be induced from zone I ectoderm by certain signals from zone IV endoderm from *either* urodeles or anurans.

Similar experiments have been reported by Maufroid and Capuron (1985) using 8-cell embryos of *Pleurodeles waltlii*. They have concluded that in *Pleurodeles* as in other urodeles, PGCs are not determined at the eight-cell stage, but subsequently arise from somatic cells following an inductive interaction with the whole endoderm.

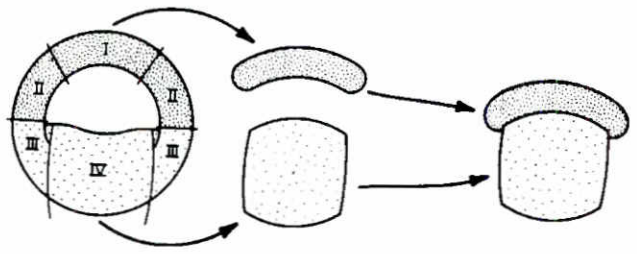

Fig. 3. Experimental scheme for the subdivision of the blastula into the four animal-vegetal zones *(after Nieuwkoop, 1969) used for the recombination of zones I and IV and subsequent culture of the recombinants in vitro.*

Morphogenetic potencies of pgc nuclei

Nuclear transplantation experiments performed with different types of anuran and urodele amphibians have shown that, except in some cases in *Xenopus laevis* (Gurdon, 1962; Gurdon and Uehlinger, 1966), somatic nuclei lose their ability to support development during embryogenesis (DiBerardino, 1980).

Similar experiments have been performed with germ cell nuclei in *Rana pipiens*: nuclei from PGCs of 11 day-old tadpoles promoted the development of larvae which could undergo metamorphosis (Smith, 1965). Furthermore, Lesimple *et al.* (1987) examined modifications of morphogenetic potencies of germ cells during the larval period in *Pleurodeles waltlii*. They demonstrated for the first time that some germ cell nuclei are able to support development into adult, fertile animals. These nuclei are, therefore, totipotent. Nevertheless, they noted a decrease in the number of hatching larvae obtained from the transplantation of germ cell nuclei taken from older larvae. This decrease could be due to modifications in, or a restriction of, the morphogenetic potencies of germ cell nuclei. From these results, it is assumed that some germ cells will be left totipotent but many become pluripotent in urodelan development.

Figure 4 provides a diagrammatic scheme of germ cell differentiation in the Urodela. After fertilization certain factors from the vegetal yolk mass induce animal cells to form mesodermal tissues (described below), and thus ectoderm, mesoderm and endoderm are established before gastrulation. Presumptive PGCs (germ cell-1: GC1 in Fig. 4) are formed in the lateral plate mesoderm and migrate into the germinal ridges during embryogenesis. Some germ cells (GC2) will behave as stem cells and be left totipotent. Many of the progenitor cells (GC3) become pluripotent and differentiate into gametes.

Future prospects for urodele germ-cell studies

Mesoderm induction by chemical messengers

During early amphibian development prospective ectodermal cells are stimulated to form mesodermal tissues by certain signals from the vegetal hemisphere (see Nieuwkoop, 1973). These signals are called mesoderm-inducing factors. Smith (1987) originally showed that a *Xenopus* cell line secreted a factor that could induce various mesodermal tissues. Many further studies have suggested that a relationship exists between mesoderm induction and several cell growth factors and hormones such as fibroblast growth factor (FGF), transforming growth factor (TGF-ß) and erythroid differentiation factor (EDF, activin A) (Fukui and Asashima, 1994).

Recall that it is now widely accepted that in urodeles PGCs do *not* develop from predetermined elements, but rather arise epigenetically from common, totipotent or pluripotent cells of the animal moiety of the blastula as part of the regional induction of the mesoderm by the vegetal yolk endoderm (Sutasurya and Nieuwkoop, 1974; Michael, 1984). Thus, it can be expected that PGCs in urodeles are induced from prospective ectodermal cells under the influence of mesoderm-inducing factors. It is of interest to remember that Kocher-Becker and Tiedemann (1971) succeeded in inducing PGCs in explants of *Triturus* ectoderm by

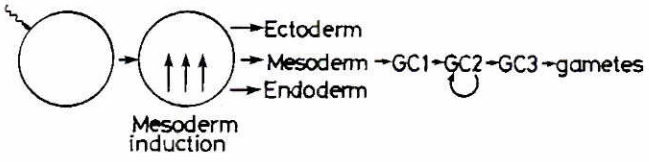

Fig. 4. A tentative scheme of germ cell differentiation in urodeles. *After mesodermal induction presumptive PGCs (GC1) are formed in the lateral plate mesoderm. Some germ cells will be left totipotent (GC2) and behave as stem cells. Many of the progenitor cells (GC3) become pluripotent and differentiate into gametes.*

adding vegetalizing factors from chick embryos. Unfortunately, however, such studies on the induction of mesodermal tissues and PGCs in urodeles by chemical messengers are limited to those of Kocher-Becker and Tiedemann. Almost all studies on mesoderm induction have been done using *Xenopus* embryos.

Activin, a peptide growth factor, can induce presumptive ectoderm from *Xenopus* blastula to differentiate into almost all mesodermal tissues in a dose-dependent fashion (Ariizumi *et al.*, 1991a,b). Furthermore, a complete set of mesodermal tissues can be induced in presumptive ectodermal explants which are treated with combinations of activin and retinoic acid of various concentrations (Moriya *et al.*, 1993). Assuming that mesoderm induction in urodeles is regulated by a similar mechanism to that in *Xenopus*, and that PGCs in urodeles are induced from the animal moiety of blastula, it should be possible to induce urodele PGCs in prospective ectodermal cells *in vitro* with combinations of chemical messengers (such as activin and retinoic acid). Studies along those lines should help solve long-standing problems of germ cell formation in urodeles.

Gene regulation of germ cell formation

Although molecules involved in germ cell formation in amphibians have not been identified, as mentioned earlier, many aspects of germ plasm formation in *Xenopus laevis* and *Rana pipiens* are strikingly similar to *Drosophila*. By isolating *Drosophila* mutants that affect germ cell formation, eight "grandchildless" genes have been identified: *cappucino*, *spire*, *staufen*, *oskar*, *vasa*, *valois*, *tudor* and *mago nashi* (see Lehmann and Ephrussi, 1994). Analysis of these genes indicates that they may act in a stepwise manner or show a genetic hierarchy. Indeed, germ plasm might be progressively assembled from that set of gene products: *cappucino*, *spire* and *staufen* may be specifically involved in the transport or anchoring of germ plasm components; *oskar*, *vasa* and *tudor* may be involved in the assembly of germ plasm components. Other genes, such as *germ-cell-less* and the *mitochondrial-large ribosomal RNA* gene represent promising candidates for guiding germ cell segregation.

Just as the extraordinary progress in the molecular biology of regulatory genes involved in pattern formation or morphogenesis in *Drosophila* (e.g., homeotic and segmentation genes) provided "breakthroughs" and made it possible to analyze "morphogenetic genes" in a variety of vertebrates, recent advances in the molecular biology of germ cell formation in *Drosophila* described above will help in analyzing and understanding the molecular basis of regulatory gene function in germ cell formation in vertebrates. For example, antibodies to *Drosophila vasa* protein revealed that a *vasa*-like protein is present in germ line cells in *Xenopus* (Watanabe *et al.*, 1992). Further, Komiya *et al.* (1994) have succeeded in isolating XVLG1 (*Xenopus vasa*-like gene), a homolog of *Drosophila vasa*), from a *Xenopus* ovary cDNA library. That data suggests that germ cell formation in *Xenopus laevis* may be regulated by molecular mechanisms similar to that employed in *Drosophila*. Until now, however, no molecular approaches have been applied to germ cell formation in urodeles.

Epigenesis vs preformation

Assuming that germ cell formation in all animals requires the expression of a series of genes which constitute a cascade and are involved in the complex gene product interactions described for *Drosophila*, the three different modes of germ cell formation (epigenetic, intermediate and preformistic) can be explained in terms of similar spatio-temporal gene-expression programs. For the sake of discussion, all the genes that are involved in germ cell formation are classified into three categories: Class I genes are speculated to function in pattern (or spatial) specification in germ cell formation (e.g., *cappucino*, *spire*, and *staufen* in *Drosophila*, and others); Class II genes are proposed to regulate the assembly of germ plasm components (e.g., *oskar*, *vasa* and *tudor* in *Drosophila*, and *XVLG1* in *Xenopus*); and Class III genes are hypothesized to control germ cell segregation from somatic cells as well

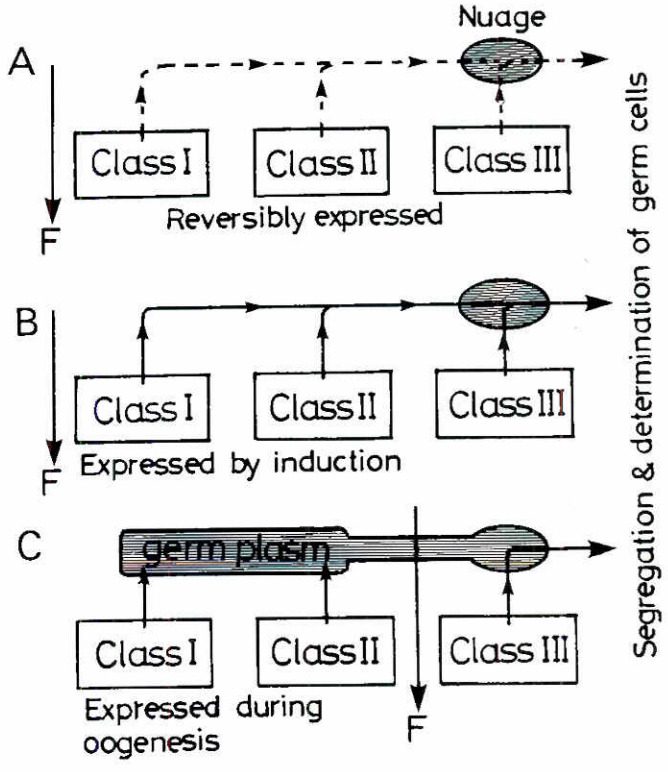

Fig. 5. A model for the role of gene expression in three different modes of germ cell formation; *epigenetic* **(A)**; *intermediate* **(B)**; *and preformistic* **(C)**. *Class I genes, which correspond to cappucino, spire and staufen in Drosophila, might function in pattern specification in germ cell formation; Class II genes, which correspond to oskar, vasa and tudor in Drosophila, might regulate the assembly of germ plasm components; and Class III genes, which correspond to the germ-cell-less and mitochondrial-large ribosomal RNA gene in Drosophila, might control germ cell segregation from the soma and then the migration of germ cells. F, fertilization. For further explanation see text.*

as the migration of germ cells (e.g., germ-cell-less, and the mitochondrial-large ribosomal RNA gene in *Drosophila* and others).

Figure 5 shows a model of gene regulation for the three different modes of germ cell formation in terms of temporal expression of those three classes of genes. The epigenetic mode of germ cell formation (Fig. 5A) is considered to result in a later and reversible expression of all the Class I, II and III genes. The presence of these genes in animals which employ this mode is supported in part by observations that even in coelenterates germ cell-specific cytoplasmic structures and *nuage* materials appear once germ cells are established (Noda and Kanai, 1977).

The preformistic mode (Fig. 5C) implies a much earlier expression of the genes. Possibly, all the genes of Classes I and II may be maternally expressed. The localization of germ plasm is considered to represent a cytoplasmic manifestation of factors which are preformed during oogenesis as a result of maternal expression of Class I and II genes. Most genes are postulated to exert instructive influences on the nucleus when presumptive germ nuclei are exposed to germ plasm. Only Class III genes are considered to be expressed during later embryogenesis.

In animals showing the intermediate mode of germ cell formation (Fig. 5B), neither Class I nor Class II genes are expressed before fertilization. Instead, they are first expressed during early embryogenesis. Because the expression of both Class I and II genes never occurs before fertilization, preformed germ plasm can be neither synthesized nor stored during oogenesis by animals with this intermediate mode. In urodeles, these genes could be expressed under the influences of certain mesodermal inducer molecules (see Fig. 4). Although the Class I and II gene products in the intermediate mode do not construct morphologically distinctive germ plasm during early embryogenesis, they are expected to exert a regulatory role for the specification of the germ line cells which is similar to the preformistic mode. Once germ cells are segregated as a result of Class III gene expression, germ cell-specific *nuage* materials, which may represent modified gene products of a part of the Class II genes, appear in their cytoplasm. Provided that Class III gene expression is irreversible during normal development, germ cells once segregated will fulfil their destiny as germ cells.

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References

AKITA, Y. and WAKAHARA, M. (1985). Cytological analyses of factors which determine the number of primordial germ cells (PGCs) in *Xenopus laevis*. *J. Embryol. Exp. Morphol. 90:* 251-265.

ARIIZUMI, T., MORIYA, N., UCHIYAMA, H. and ASASHIMA, M. (1991a). Concentration-dependent inducing activity of activin A. *Roux Arch. Dev. Biol. 200:* 230-233.

ARIIZUMI, T., SAWANUMA, K., UCHIYAMA, H. and ASASHIMA, M. (1991b). Dose and time dependent mesoderm induction and out-growth formation by activin A in *Xenopus laevis*. *Int. J. Dev. Biol. 35:* 407-411.

BLACKLER, A.W. (1958). Contribution to the study of germ-cells in the Anura. *J. Embryol. Exp. Morphol. 6:* 491-503.

BLACKLER, A.W. (1966). Embryonic sex cells in Amphibia. *Adv. Reprod. Physiol. 1:* 9-28.

BLACKLER, A.W. (1970). The integrity of the reproductive cell line in the Amphibia. *Curr. Top. Dev. Biol. 5:* 71-87.

CLARK, J.M. and EDDY, E.M. (1975). Fine structural observations on the origin and associations of primordial germ cells of the mouse. *Dev. Biol. 47:* 136-155.

CZOLOWSKA, R. (1969). Observations on the origin of the 'germinal cytoplasm' in *Xenopus laevis*. *J. Embryol. Exp. Morphol. 22:* 229-251.

DIBERARDINO, M.A. (1980). Genetic stability and modulation of metazoan nuclei transplanted into eggs and oocytes. *Differentiation 17:* 17-30.

DIXON, K.E. (1981). The origin of the primordial germ cells in amphibia. *Neth. J. Zool. 31:* 5-37.

EDDY, E.M. (1975). Germ plasm and the differentiation of the germ cell line. *Int. Rev. Cytol. 43:* 229-280.

FUKUI, A. and ASASHIMA, M. (1994). Control of cell differentiation and morphogenesis in amphibian development. *Int. J. Dev. Biol. 38:* 257-266.

GINSBURG, M. (1994). Primordial germ cell formation in birds. In *Germline Development*. Ciba Foundation Symposium 182. John Wiley and Sons, Chichester, pp. 52-67.

GURDON, J.B. (1962). The developmental capacity of nuclei from intestinal epithelium cells of feeding tadpoles. *J. Embryol. Exp. Morphol. 10:* 622-640.

GURDON, J.B. and UEHLINGER, V. (1966). "Fertile" intestine nuclei. *Nature 210:* 1240-1241.

HAMASHIMA, N. and KOTANI, M. (1977). Ultrastructural observations on the primordial germ cells in the newt, *Triturus pyrrhogaster*. *Zool. Mag. 86:* 239-245.

HAMASHIMA, N. and KOTANI, M. (1979). '*Nuage*'-chromosome association in metaphase spermatogonia of the newt, *Cynops pyrrhogaster*. *Dev. Growth Differ. 21:* 221-227.

HEASMAN, J., QUARMBY, J. and WYLIE, C.C. (1984). The mitochondrial cloud of *Xenopus* oocytes: the source of germinal granule material. *Dev. Biol. 105:* 458-469.

HUMPHREY, R.R. (1925). The primordial germ cells of *Hemidactylium* and other Amphibia. *J. Morph. Physiol. 41:* 1-43.

HUMPHREY, R.R. (1927). Extirpation of the primordial germ cells in *Amblystoma:* its effect upon the development of the gonad. *J. Exp. Zool. 49:* 363-399.

HUMPHREY, R.R. (1928). The developmental potencies of the intermediate mesoderm of *Amblystoma* when transplanted into ventro-lateral sides on other embryos: the primordial germ cells of such grafts and their role in the development of gonads. *Anat. Rec. 40:* 67-90.

HUMPHREY, R.R. (1929). The early position of the primordial germ cells in urodeles: evidence from experimental studies. *Anat. Rec. 42:* 301-314.

IKENISHI, K. and KOTANI, M. (1975). Ultrastructure of the 'germinal plasm' in *Xenopus* embryos after cleavage. *Dev. Growth Differ. 17:* 101-110.

IKENISHI, K. and NIEUWKOOP, P.D. (1978). Localization and ultrastructure of primordial germ cells (PGCs) in *Ambystoma mexicanum*. *Dev. Growth Differ. 20:* 1-9.

IKENISHI, K., NAKAZATO, S. and OKUDA, T. (1986). Direct evidence for the presence of germ cell determinant in vegetal pole cytoplasm of *Xenopus laevis* and in a subcellular fraction of it. *Dev. Growth Differ. 28:* 563-568.

KAMIMURA, M., IKENISHI, K., KOTANI, M. and MATSUNO, T. (1976). Observations on the migration and proliferation of gonocytes in *Xenopus laevis*. *J. Embryol. Exp. Morphol. 36:* 197-207.

KOCHER-BECKER, U. and TIEDEMANN, H. (1971). Induction of mesodermal and endodermal structures and primordial germ cells in *Triturus* ectoderm by a vegetalizing factor from chick embryos. *Nature 233:* 65-66.

KOMIYA, T., ITOH, K., IKENISHI, K. and FURUSAWA, M. (1994). Isolation and characterization of a novel gene of the DEAD box protein family which is specifically expressed in germ cells of *Xenopus laevis*. *Dev. Biol. 162:* 354-363.

KOTANI, M. (1957). On the formation of primordial germ cells from the presumptive ectoderm of *Triturus* gastrulae. *J. Inst. Polytech. Osaka City Univ. Ser. D 8:* 145-159.

KOTANI, M. (1958). The formation of germ cells after extirpation of the presumptive lateral plate mesoderm of *Triturus* gastrulae. *J. Inst. Polytech. Osaka City Univ. Ser. D 9:* 195-209.

LAWSON, K.A. and HAGE, W.J. (1994). Clonal analysis of the origin of primordial germ cells in the mouse. In *Germline Development*.. Ciba Foundation Symposium 182. John Wiley and Sons, Chichester, pp. 68-91.

LEHMANN, R. and EPHRUSSI, A. (1994). Germ plasm formation and germ cell determination in *Drosophila*. In *Germline Development*. Ciba Foundation Symposium 182. John Wiley and Sons, Chichester, pp. 282-300.

LESIMPLE, M., DOURNON, C. LABROUSSE, M. and HOUILLON, C. (1987). Production of fertile salamanders by transfer of germ cell nuclei into eggs. *Development 100:* 471-477.

MAUFROID, J.P. and CAPURON, A. (1985). A demonstration of cellular interactions during the formation of mesoderm and primordial germ cells in *Pleurodeles waltlii*. *Differentiation 29:* 20-24.

MCKAY, D.G., HERTIG, A.T., ADAMS, E.C. and GANZIGER, S. (1953). Histochemical observations on the germ cells of human embryos. *Anat. Rec. 117:* 201-219.

MICHAEL, P. (1984). Are the primordial germ cells (PGCs) in Urodela formed by the inductive action of the vegetative yolk mass? *Dev. Biol. 103:* 109-116.

MILAIRE, J. (1957). Contribution `a la connaissance morphologique et cytochimique de bourgeons de mebres chez quelques reptiles. *Arch. Biol. 68:* 429-512.

MORIYA, N., UCHIYAMA, H. and ASASHIMA, M. (1993). Induction of pronephric tubules by activin and retinoic acid in presumptive ectoderm of *Xenopus laevis*. *Dev. Growth Differ. 35:* 123-128.

NIEUWKOOP, P.D. (1947). Experimental investigations on the origin and determination of the germ cells, and on the development of the lateral plates and germ ridges in the urodeles. *Arch. Neerl. Zool. 8:* 1-205.

NIEUWKOOP, P.D. (1969). The formation of the mesoderm in urodelan amphibia. I. Induction by endoderm. *W. Roux Arch. Entw.Mech. 162:* 341-373.

NIEUWKOOP, P.D. (1973). The 'organization centre' of the amphibian embryo: its origin, spatial organization and morphogenetic action. *Adv. Morphogen. 10:* 1-39.

NIEUWKOOP, P.D. and SUTASURYA, L.A. (1979). *Primordial Germ Cells in the Chordates*. Cambridge Univ. Press, Cambridge.

NIEUWKOOP, P.D. and SUTASURYA, L.A. (1981). *Primordial Germ Cells in the Invertebrates*. Cambridge Univ. Press, Cambridge.

NODA, K. and KANAI, C. (1977). An ultrastructural observation on *Pelmatohydra robusta* at sexual and asexual stages, with reference to 'germinal plasm'. *Ultrastruct. Res. 61:* 284-294.

SCOTT, M.P. (1994). Intimations of a creature. *Cell 79:* 1121-1124.

SMITH, J.C. (1987). A mesoderm-inducing factor is produced by a *Xenopus* cell line. *Development 99:* 3-14.

SMITH, L.D. (1965). Transplantation of the nuclei of primordial germ cells into enucleated eggs of *Rana pipiens*. *Proc. Natl. Acad. Sci. USA 54:* 101-107.

SMITH, L.D., MICHAEL, P. and WILLIAMS, M.A. (1983). Does a predetermined germ line exist in amphibians? In *Current Problems in Germ Cell Differentiation* (Eds. by A. McLaren and C.C. Wylie). Cambridge Univ. Press, Cambridge, pp. 19-39.

SUTASURYA, L.A. and NIEUWKOOP, P.D. (1974). The induction of the primordial germ cells in the urodeles. *W. Roux Arch. Entw.Mech. 175:* 199-220.

WAKAHARA, M. (1977). Partial characterization of 'primordial germ cell-forming activity' localized in vegetal pole cytoplasm in anuran egg. *J. Embryol. Exp. Morphol. 39:* 221-233.

WAKAHARA, M. (1978). Induction of supernumerary primordial germ cells by injecting vegetal pole cytoplasm into *Xenopus* eggs. *J. Exp. Zool. 203:* 159-164.

WAKAHARA, M. (1990a). Germ plasm: its role in species continuity on the earth and in space. In *Foundation of Space Biology* (Eds. M. Asashima and G.M. Malacinski). Japan Sci. Soc. Press, Tokyo, pp. 139-151.

WAKAHARA, M. (1990b). Cytoplasmic localization and organization of germ-cell determinants. In *Cytoplasmic Organization System* (Ed. G.M. Malacinski). McGraw-Hill Publ. Co., New York, pp. 219-242.

WATANABE, M., ITOH, K., ABE, K., AKIZAWA, T., IKENISHI, K. and FURUSAWA, M. (1992). Immuno-localization of DEAD family proteins in germ line cells of *Xenopus* embryos. *Dev. Growth Differ. 34:* 223-231.

WHITINGTON, P. and DIXON, K.E. (1975). Quantitative studies of germ plasm and germ cells during early embryogenesis of *Xenopus laevis*. *J. Embryol. Exp. Morphol. 33:* 57-74.

WILLIAMS, M.A. and SMITH, L.D. (1971). Ultrastructure of the 'germinal plasm' during maturation and early cleavage in *Rana pipiens*. *Dev. Biol. 25:* 568-580.