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

MASAMI WAKAHARA*

Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo, Japan

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

*Address for reprints: Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060, Japan. FAX: 11.7575994, e-mail: chami@bio.hokudai.ac.jp
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 Urodeles (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 Urodeles 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 urodeles

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 (Czołowska, 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; Whittington 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 extragondadal 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 extragondadal PGCs of reptiles (Milaire, 1957).

As described above, presumptive PGCs of urodeles have no specific markers for their identification, while many groups of other chordates show certain morphological or cytochemical features specific to the presumptive PGCs or extragondadal 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 egg 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 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 urodeles**

**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-stripe 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 lead 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 origin.

**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 anuran), they migrate to and eventually reach the germinal ridges at the swimming stage.
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.
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 (Arizumi 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 outstanding problems of germ cell formation in urodeles.

**Gene regulation of germ cell formation**

Although molecules involved in germ cell formation in amphibia 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 "morpheogenetic 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.
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 urodèles, 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 fulfill their destiny as germ cells.

Acknowledgments

I would like to thank G.M. Malacinski for his critical reading of the manuscript and for valuable discussions, and Susan Duhon for editorial assistance.

References


