Primordial Germ Cell Development in Avians

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ABSTRACT The origin of the germline is studied in avians by tracing primordial germ cells from the stage of the germinal crescent backwards to earlier developmental stages. It has been demonstrated that during primitive streak formation, the germline has already been segregated. However, during this stage, the cells are seen gradually migrating from the epiblast to the hypoblast. The vertical migration is followed by a horizontal translocation to the extra-embryonic germinal crescent, being carried out by the hypoblast that is pushed anteriorly by the invading endodermal cells. In contrast, it has been shown that in the mouse the germ cells are allocated during gastrulation in a cluster of cells. Our results demonstrate that in avians the allocation takes place according to a different mode. The close association between the germ cells and the extra-embryonic mesoderm indicates that the germline in avians, as in the mouse, develops from a subset of cells that have already segregated from the epiblast as extra-embryonic mesoderm.

(Key words: primordial germ cells, avian blastoderm, avian gastrulation, extra-embryonic mesoderm)

INTRODUCTION

Where and when does the germline arise in the avians? Is it set aside immediately, before embryonic cells become committed along specific pathways, or does it appear de novo from other somatic cells at some point during development? Weismann’s (1885) idea about the continuity of the germ plasma, hence, a “preformistic” mode of germ cell development, has been the paradigm for vertebrate investigators in the past.

However, new technologies have paved the way to a different analysis, demonstrating that, at least in the mouse (Lawson and Hage, 1994), the germ cells differentiate from a somatic lineage. For elucidating the mode of the germ line allocation in the other vertebrates, a detailed analysis is needed, defining the primary population of the germ cells, its location, and involvement with other developmental events.

In the avian embryos, no germplasma has ever been found. The germ cells are detected relatively late in development, toward the end of gastrulation, in an extra-embryonic area. Since they were first detected by Swift in 1914, the study of their earlier history has been very limited. Nevertheless, a wealth of factual information has accumulated during the last decade and there has been some success in tracing primordial germ cells backwards to earlier developmental stages. Elucidating the events concerned with germ cell determination and differentiation would allow the study of the molecular mechanisms that enable germ cells to give rise to the next generation.

THE PERIOD OF PRIMITIVE STREAK FORMATION

Primordial germ cells were first detected in the chicken embryos by Swift (1914) at the end of the gastrulation process. Nevertheless, they are better distinguished at the 10-somite stage as a dispersed population of approximately 200 cells located within an extra-embryonic area, the germinal crescent, anterior to the end of the embryo. At this stage, the cells are characterized by their size, by the eccentric position of their large nucleus, and by their large glycogen content, thus allowing their detection by histochemical methods such as the periodic acid Schiff staining (Meyer, 1960). From this area, they migrate into the embryo with the circulating blood.

Considering the area in which the detection has been made, two questions arise. 1) Do the primordial germ cells form at the germinal crescent or are they formed earlier elsewhere? 2) Does the formation or the migration of the primordial germ cells depend upon the gastrulation process?

During gastrulation, cells are seen leaving the epiblast through the primitive streak to form the middle layer, the mesoderm, as well as replacing the hypoblast with endoderm proper (Nicholet, 1971). As a result, the system is transformed from a two- into a three-layered system. Consequently, the hypoblast, invaded by the endoderm, is pushed anteriorly to become an extra-
are means of 7 to 10 embryos. Embryo were counted and the percentages calculated. Data presented primordial germ cells detected in each pair of layers from the same layers. These were incubated to complete 48 h of incubation. The 1951). Embryos at each stage were separated into lower and upper (Eyal-Giladi and Kochav, 1976) to Stage 4 (Hamburger and Hamilton, 1951). The upper and the lower layers of blastoderms at Stage XII to XIII (Hamburger and Hamilton, 1951). Embryos at each stage were separated into lower and upper layers. These were incubated to complete 48 h of incubation. The primordial germ cells detected in each pair of layers from the same embryo were counted and the percentages calculated. Data presented are means of 7 to 10 embryos.

FIGURE 1. Vertical distribution of primordial germ cells between the upper and the lower layers of blastoderms at Stage XII to XIII (Eyal-Giladi and Kochav, 1976) to Stage 4 (Hamburger and Hamilton, 1951). Embryos at each stage were separated into lower and upper layers. These were incubated to complete 48 h of incubation. The primordial germ cells detected in each pair of layers from the same embryo were counted and the percentages calculated. Data presented are means of 7 to 10 embryos.

embryonic tissue (Vakaet, 1962). This area constitutes the germinal crescent, where the primordial germ cells are located. Moreover, an extra-embryonic mesoderm that gives rise to extra-embryonic blood circulation is always associated with the primordial germ cells in this area. Because reliable specific markers for early avian primordial germ cells are not yet available, our ability to trace their history is limited to strategies that rely mainly on morphological criteria. Hence, the various manipulations that have been performed on early gastrulating embryos could be analyzed only after further incubation for a period equivalent to that which allowed a control blastoderm to reach Stage 10 (Hamburger and Hamilton, 1951).

Separating the lower layer from embryos at stages of primitive streak formation and culturing it in parallel with the embryos from which it was taken (Ginsburg and Eyal-Giladi, 1986) has demonstrated that 1) The primordial germ cell population at the germinal crescent increases during gastrulation due to migration of additional cells from the lower layer. 2) The removal of the hypoblast prevents this additional population from reaching the germinal crescent. 3) The migration is a gradual process: the later the stage at which the separation was performed, the larger the proportion of the primordial germ cell population that appeared in the lower layer (Figure 1). Because the hypoblast moves anteriorly to become the major component of the germinal crescent, it could serve as a vehicle for the translocation of the primordial germ cells into the extra-embryonic area.

We have used a polyclonal antiserum produced against quail gonadal primordial germ cells for a direct and specific detection of primordial germ cells in the quail hypoblast during gastrulation (Ginsburg et al., 1989). Using this technique, our observations indicate that: 1) In avians, the germline exists as a separate lineage during primitive streak formation; 2) The primordial germ cells are carried into the germinal crescent mainly by the hypoblast; 3) The translocation into the extra-embryonic area is gradual and terminates only at the end of gastrulation.

**THE PERIOD OF HYPOBLAST FORMATION**

The hypoblast is the ventral layer of the blastoderm. It starts to form as individual cells ingress from the ventral surface of the one-layered area pellucida and spread as islands beneath this surface. It gradually merges into a continuous layer, into which a front of cells, advancing from the posterior marginal zone, is integrated (Weinberger and Brick, 1982). The presence of primordial germ cells in the hypoblast has led some investigators to conclude that the primordial germ cells originate in the hypoblast (Dubois, 1969). However, Eyal-Giladi et al. (1981), investigating chimeras of quail hypoblast and chicken epiblast (or vice versa), established that the avian primordial germ cells are of epiblastic origin. This finding must mean that they migrate to the hypoblast during development. However, the close association between the hypoblast and the primordial germ cells, which has not yet been analyzed, may reveal that the growing hypoblast is more significant to the development of the germ cells than that of a mere vehicle of translocation.

The presence of a few primordial germ cells under the area pellucida at Stages X to XIII (Eyal-Giladi and Kochav, 1976), was reported by several investigators who used various antibodies, none of which were specific for avian primordial germ cells (Urven et al., 1988, with EMA-1; Pardanaud et al., 1987, with QH1 in the quail; Loveless et al., 1990, with FC10.2). However, even if the labeled cells were in fact primordial germ cells, immunohistochemistry could detect only those that had already segregated. Hence, the existence and distribution of the primordial germ cell progenitors within the area pellucida at those developmental stages remains obscure. Dissecting blastoderms at Stages X to XII to small fragments and allowing those fragments to grow in culture for 2 d as tiny explants (Ginsburg and Eyal-Giladi, 1987) demonstrated the presence of primordial germ cells in all the fragments that originated from the area pellucida. No primordial germ cells could be detected in fragments that were excised from the area opaca. As was demonstrated in whole mounts preparations stained with periodic acid Schiff and in histological sections each tiny explant derived from the area...
pellicula has developed a lower layer. The primordial germ cells were found scattered within a middle layer comprised of mesenchyme-like cells, which start to form the blood system. The total number of primordial germ cells counted in all pieces derived from the same blastoderm was always in the same range (150 to 200) as found in control blastoderms incubated for 2 d.

At this stage of the investigation, the following models were considered (Figure 2): 1) Primordial germ cells progenitors are sparsely located in the area pellicula already at Stage X. 2) There is no lineage restriction to the germline at Stage X. However, a stochastic event transmits a signal to the pluripotent epiblast cells, causing some of them to differentiate into primordial germ cells. 3) The differentiation into primordial germ cells is induced within a subpopulation of cells that has already been segregated from the epiblast.

CIRCUMVENTING THE PRIMITIVE STREAK

Isolation of the area pellicula from the area opaca and the subsequent dissociation and culture of the area pellicula-derived cells as an aggregate (Ginsburg and Eyal-Giladi, 1989) would not support gastrulation because polarity was destroyed. However, the expected number of primordial germ cells exist and they are located between the mesenchyme-like tissue, which constitutes the middle layer, and the ventral layer. This result implies that three lineages do not need the primitive streak for their differentiation: the hypoblast, the mesenchyme-like mesoderm that develops into blood tissue, and the primordial germ cells. These three lineages also do not require the area opaca because this had been previously discarded.

The observed association of the avian primordial germ cells with the mesenchyme cells that are not part of the gastrulated axial mesoderm has focused our attention on the extra-embryonic mesoderm, a cell lineage that has been rather neglected in studies of early embryogenesis of the avians. In all the various manipulations that we have performed on early stages, primordial germ cells were always associated with an extra-embryonic mesoderm. Support for our observation came from a recent study in the mouse (Lawson and Hage, 1994). By labeling cells in the proximal epiblast in both pregastrulation and early-streak embryos, these investigators have demonstrated that the precursors of the primordial germ cells constitute a part of the presumptive extra-embryonic mesoderm and are not lineage-restricted while in the epiblast. They are allo-
cated during gastrulation as a cluster of approximately 40 cells already segregated in the extra-embryonic mesoderm posterior to the primitive streak. The meaning of this observation is that during gastrulation in the mouse embryo, cells are segregating from the epiblast, circumventing the primitive streak, to form the extra-embryonic mesoderm. The descendants of this lineage that are located in a specific area at the base of the allantois are differentiated by an unknown mechanism into germ cells.

In the avian embryo, no cluster of primordial germ cells has ever been found. Compared to the mouse, the avian germline already exists as a separate lineage of scattered cells prior to primitive streak formation. We hypothesize that in the avian embryo, the extra-embryonic mesoderm segregates from the epiblast earlier than the formation of the primitive streak. We assume that the first cells that leave the ventral surface of the area pellucida form clusters that spread and merge to form the hypoblast (Figure 3). However, cell ingression is continuous and results in the accumulation of additional cells between the epiblast and the hypoblast. This population of cells constitutes the extra-embryonic mesoderm from which some cells differentiate into the germ line. Being located on the hypoblast, both the extra-embryonic mesoderm and the primordial germ cells are later carried anteriorly, away from the gastrulating area.

There is evidence in avians for a middle layer of cells between epiblast and hypoblast prior to primitive streak formation (Izpisua-Belmonte et al., 1993; Ruiz i. Altaba et al., 1995). Hatada and Stern (1994) have demonstrated by carbocyanine dyes labeling, that cells contributing to the yolk sac and its stalk are very widely distributed over the entire surface of the prestreak blastoderm. Nevertheless, no detailed cell lineage analysis for the extra-embryonic mesoderm has ever been performed in avians. We therefore think that the location of the presumptive extra-embryonic mesoderm as being described by Stern (1992), in the fate map for Stages X to XIII at the posterior end of the blastoderm, should be revised.

Does the germline in the avians segregate from the epiblast earlier than in the mouse? Izpisua-Belmonte et al. (1993) have demonstrated an early small population of middle layer cells, associated with Koller’s sickle, that express the gene goosecoid during stages of hypoblast formation. If the expression of goosecoid is indeed a marker for an organizer, it means that the process of gastrulation starts in avians earlier than the visible appearance of the primitive streak. Based on these criteria, it is most likely that the segregation of the avian germline takes place, similar to the mouse, during early gastrulation.

In both the mouse embryo and in avians, the primordial germ cells seem to escape the developing primitive streak, circumventing it in two different modes. In the mouse they segregate as a cluster of cells within an extra-embryonic area posterior to the primitive streak, and only later start to populate the embryo (Ginsburg et al., 1990). However, in avians these cells segregate from the entire surface of the epiblast in a scattered mode and translocate onto an extra-embryonic tissue, the hypoblast. The latter carries them anteriorly, into an extra-embryonic location, away from the primitive streak that starts to advance from the posterior area of the blastoderm.

In a study that is currently underway, we are attempting to analyze the different populations of cells that are found between the epiblast and the hypoblast of blastoderms at Stages X to XIII. For this purpose we are using various monoclonal antibodies labeling and carbocyanine dyes labeling. We hope that a detailed analysis
of the segregation mode of the extra-embryonic mesoderm from the epiblast, and the detection of the primordial germ cells within this tissue, will allow us to define the exact location and association of the germline allocation in the avians.

REFERENCES