



Saga of the Sex Cells

Introduction

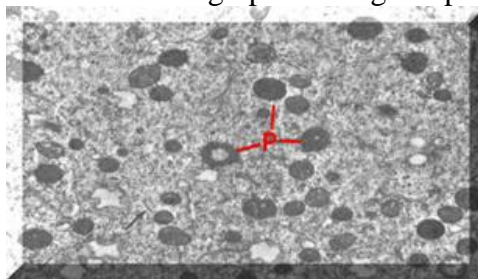
The primordial germ cells give rise to the male and female sex cells which in turn transmit the genome from generation to generation. The “Saga of the Sex Cells” is a true tale of cellular struggle, long journeys and tragic events. The future eggs and sperm are critical to the survival of each species. As a result, in some species, their fate is determined long before the egg begins to divide. Special factors (determinants) dictate that the cells that contain them will become sex cells. The cells that acquire these determinants then have a long haul ahead. First they must migrate through the embryonic tissues to the sites where the future gonads will form. Along the way some will get lost and simply die. Other lost cells will not accept their inability to get to where they were supposed to and will begin to develop inappropriately forming dangerous malignant tumors called teratomas. Those that do arrive safely will have to wait many long years as the genital ridges first develop into gonads and then, at puberty, become functional producers of mature gametes. In the ovaries, a limited supply of eggs will be produced that will have to suffice for the life of the female while in the male, sperm production will be a continuous process that can last until death. The way in which eggs and sperm form and the differences in their formation are equally exciting and reflect the important role each has in ensuring that successful fertilization will result so that the next generation will begin.

What are Primordial Germ Cells (PGCs)?

- PGCs are the precursors (i.e., a type of stem cell) of the sex cells
- In lower animals, PGCs are determined by germ plasm
- True germ plasm may not exist in mammals
- Similar genes to those found in lower animals appear to mediate human PGC formation
- Mammalian PGCs arise just prior to gastrulation
- They first appear in the epiblast in the region that will become the extraembryonic mesoderm
- Alkaline phosphatase is a germ cell "marker enzyme" for PGCs
- Since they arise extra-gonadally, PGCs must migrate through different tissues to reach the genital ridges

What is Germ Plasm?

Just what does the germ plasm look like? Does it look like any other region of the cytoplasm or is it specialized in some way? Below is an electron micrograph of the germ plasm of *Drosophila*.

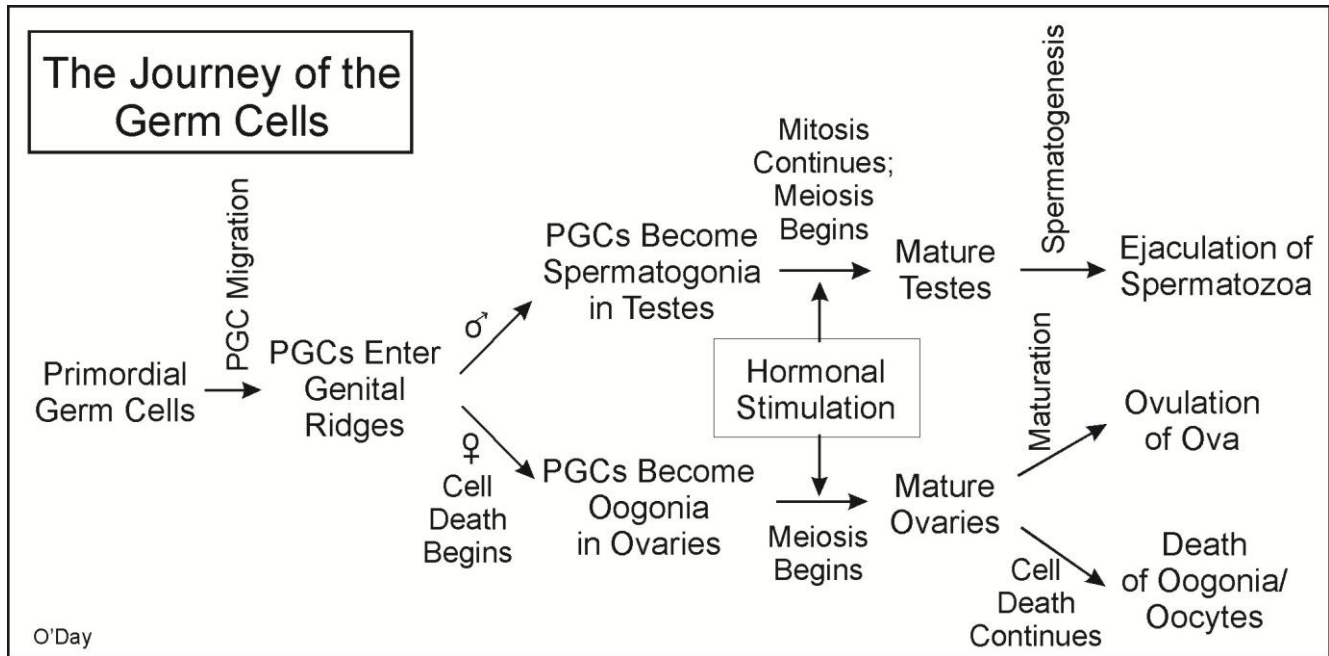


- The germ plasm is morphologically similar in all lower animals but has not been detected in mammals
- It contains dense fibrillar and granular material
- The particles have been shown to contain RNA and protein plus mitochondria and ribosomes
- It segregates into certain cells during cleavage
- Cells that receive the germ plasm are destined to become germ-line cells

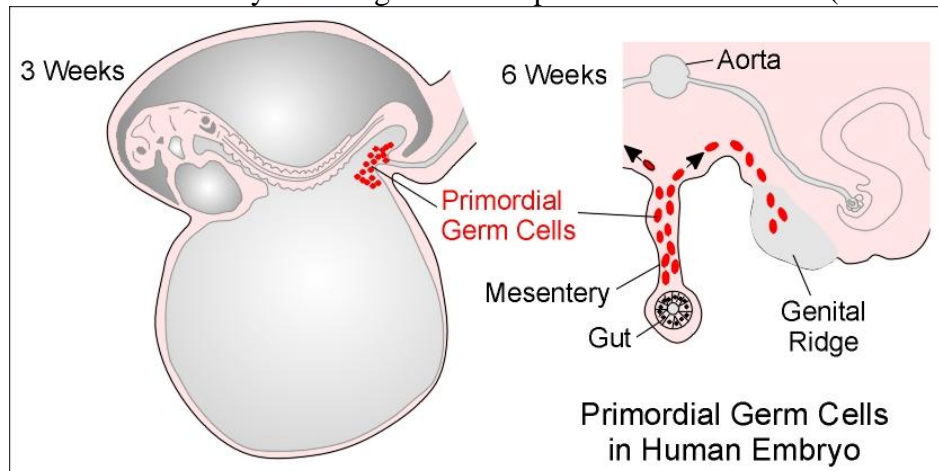
Saga of the Sex Cells

- Extensive research has been done on *Drosophila* and other organisms (e.g., *Caenorhabditis*) which has helped the study of germ line determination in humans.
- The determinants have been found to be genetically controlled
- Specific mRNAs appear to be the cytoplasmic determinants that underlie the formation of germ cells.

The Journey of the Germ Cells

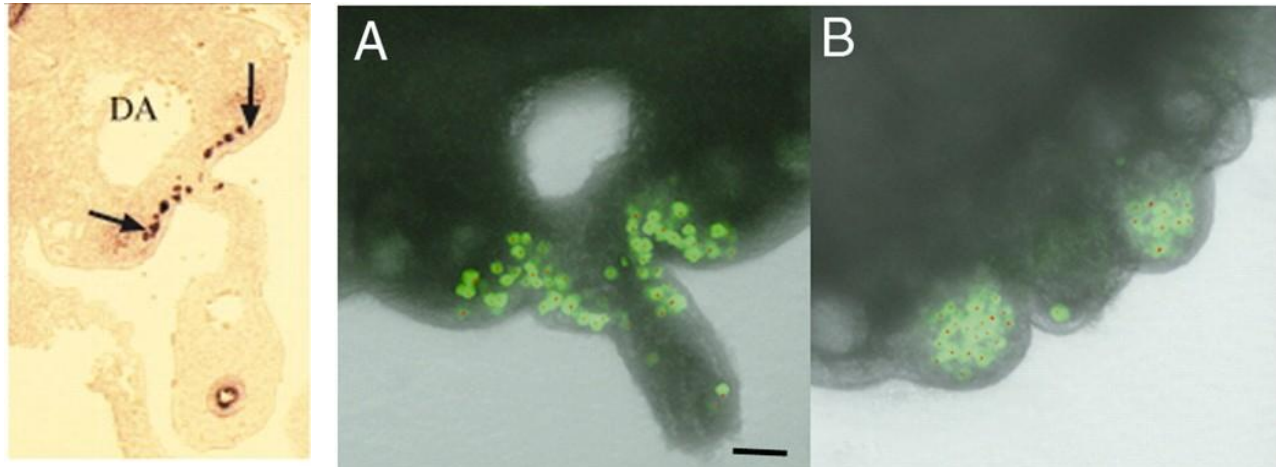


- The PGCs migrate from their site of origin in the epiblast to the future genital ridges
- During these early stages the number of PGCs increases by mitotic division
- In the genital ridges they will come under various influences (i.e., hormones, cellular interactions) that will dictate that they will become either oogonia (females) or spermatogonia (males)
- In the female genital ridges, cell death of the PGCs will begin and continue; mitosis does not continue
- In the male genital ridges, mitosis of the PGCs will continue; cell death is minimal
- The hormonal events of puberty will cause the PGCs to complete their differentiation
- Gametogenesis continues until fully formed gametes are produced and released (ovulation or ejaculation)



Saga of the Sex Cells

Early work using alkaline phosphatase staining, a germ cell marker enzyme, has been used to reveal the pattern of germ cell migration in various mammals including humans. More recently cellular labeling (e.g., GFP; green fluorescent protein linked to a germ cell specific protein) and use of staining with monoclonal antibodies directed against germ cells has added further insight into this subject. For example, Stromal cell derived factor-1 (SDF-1) and its chemokine receptor CXCR-4 are essential for germ cell migration.



Alkaline Phosphatase

GFP-SDF1 (Stromal Cell Derived Factor-1)

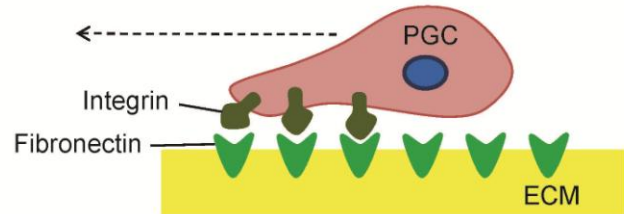
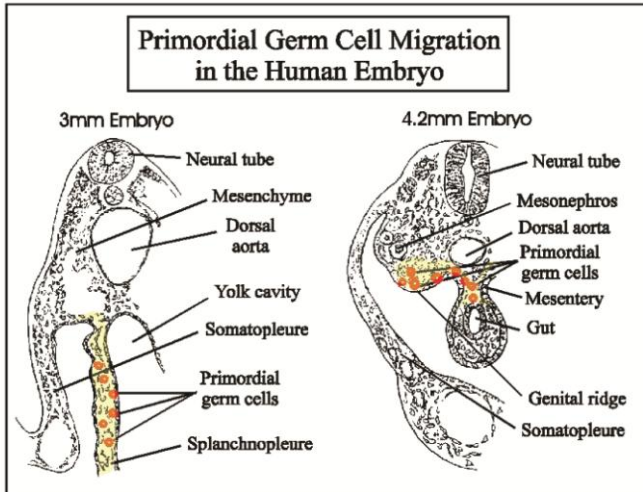
Figures courtesy of Kathleen Molyneaux (Molyneaux et al, 2003. *Development* 130: 4279-4286).

As detailed below, transplantation studies have shown that the germ cell lineage begins in the posterior region of the epiblast in the mouse. The above picture shows that PGCs are subsequently detected in the yolk sac, a long distance--in cellular terms--from the future ovaries and testes. They migrate up the mesentery (splanchnopleure) ultimately exiting left & right to enter the just forming genital ridges. Always remember, that as these events are happening the embryo is changing continuously so something that was present early in the process may not be present later or may have changed in its organization and appearance.

How Do the PGCs Know Where to Go?

There is evidence that the genital ridges may secrete some chemoattractant that guides the PGCs towards them. PGCs migrate by extending filopodia (fine pseudopods) and they can migrate between cells in tissues. In addition, they may follow extracellular matrix components that serve as "roadways" leading to the genital ridges. These roadways are lined with fibronectin. There is some evidence that the integrins are involved in the migration of PGCs since mutants lacking integrins fail to migrate into the gonads. Integrins are localized to the surface of cells where they act as receptors for molecules (e.g., fibronectin) in the ECM.

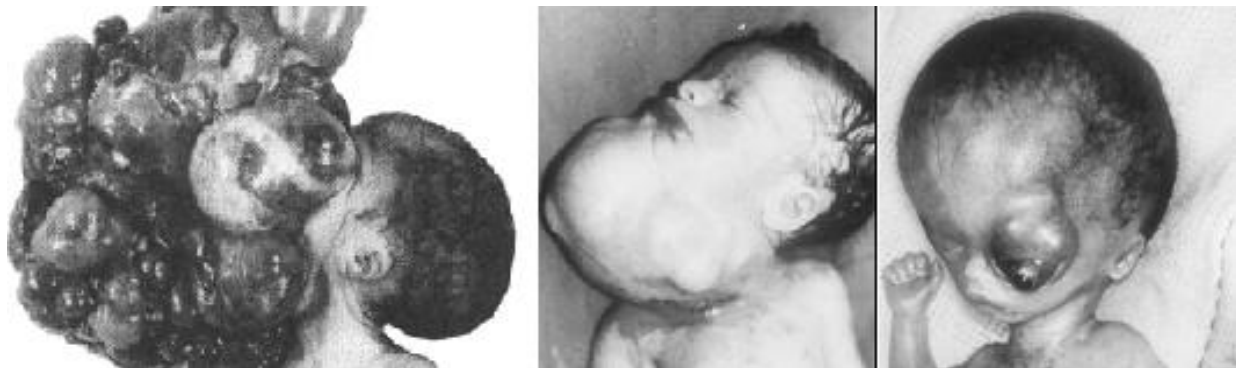
Below (in the left-hand panel of the figure) is a completely theoretical demonstration of how PGCs (red) might use their surface integrins to follow an embryonic "roadway" of extracellular material to reach the genital ridges. The yellow is meant to reflect a theoretical complex of ECM components (e.g., laminin, fibronectin, collagen) rather than a single entity such as fibronectin. Cells would stay within the yellow ECM region rather than wander into adjacent regions because they would have preferential adhesiveness ("stickiness") to the yellow "roadway". The right-hand image shows a primordial germ cell moving along the ECM via binding of the cell adhesion molecule integrin to fibronectin in the ECM.



PGC migration is also controlled by TGF-beta 1 which is secreted by the genital ridge. TGF β 1 acts as a chemoattractant but inhibits proliferation, thereby modulating the number of primary germ cells in the gonad. The topics of chemotaxis and extracellular "roadways" will be discussed in more detail in future lectures especially when we detail the migration of Neural Crest cells.

Teratomas

- Cancerous masses containing differentiated cells that are in a disorganized state
- Teratomas look like tiny disorganized embryos
- They arise due to PGCs getting "lost" in non-gonadal sites
- Because of their "totipotent" nature, PGCs can differentiate into diverse cell types
- Thus, teratomas can contain hair, skin, cartilage, teeth, etc.



Images by: Mason Barr, Ann Arbor, MI and others

The disorganized state of the teratoma is believed to be a result of "lost" PGCs ending up in embryonic locales where they fail to get the proper signals for development. Since the PGCs are totipotent--they have the ability to differentiate into all of the cells of the human body--they differentiate into diverse cell types. But their organization is haphazard because they don't get the proper information to organize into the embryonic pattern. The "totipotent" nature of primordial germ cells and their ability to function properly has been shown more directly by transplantation experiments. When cells from teratomas (from one genetic strain) are inserted into the inner cell mass of normal mouse embryos (from another genetic strain), mouse teratoma cells contribute normally to development. Instead of a mouse full of malignant teratomas, a normal healthy mouse is formed. Genetic analyses verified that the cells of the teratoma were present in the normal

tissues. This elegant experiment verifies the totipotency of these cells and their ability to develop appropriately given the right signals. While PGCs are totipotent, it is likely that most stem cells are pluripotent (i.e., can form a large number of different cell types but not all of those found in the human body). This is because such stem cells (e.g., from blood forming tissues, skin, etc.) have already embarked on a developmental pathway that limits their fate.

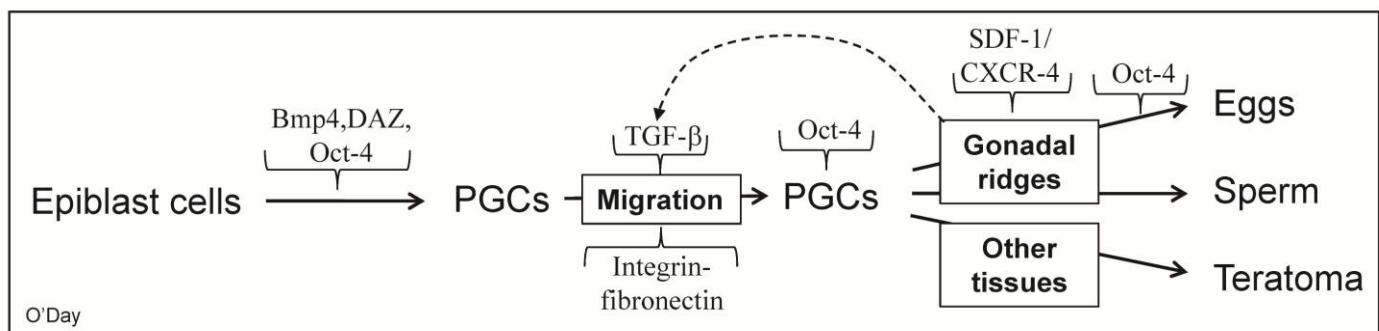
Germ Cell Formation in Mammals

In humans the PGCs, the germ cell lineage is not established in the same way as in many lower animals. For one thing, "germ plasm" does not appear to exist in mammals and the germ line is not predetermined. In the rat and mouse a similar material called "nuage" appears in germ cells but could not be detected earlier in the embryo. PGCs are derived from the posterior epiblast but transplantation experiments have shown that this material is not determined early. For example, in the mouse the germ cell lineage only becomes defined around the time of gastrulation not during oogenesis or early cleavage as it does in lower animals. Grafting experiments have shown that many regions of the mouse embryo are capable of forming germ cells when they are transplanted to the extraembryonic mesoderm region of the epiblast prior to gastrulation. Until more is known, it is assumed the human germ cell population arises in a similar way. The topics of the important topic of determination of cells and tissues and cellular interactions (e.g., induction) that mediate the process will be covered at many times throughout this course.

BMP & DAZ Genes & Human Germ Cell Formation

Various factors seem to be important such as bone morphogenetic protein (BMP; originally revealed as a factor involved in bone morphogenesis) since mice with null mutations for *Bmp4* lack primordial germ cells. While germ-line determination is likely to differ from other animals in many specific ways, work on lower forms has guided the direction of human studies. For example, over the last few years, another gene first identified in *Drosophila* as being important in germ cell development has also been shown to function in germ cell formation in humans. Mutations in the human DAZ gene (Deleted in Azoospermia) and/or its homologs can result in the absence of either eggs or sperm cells. The exact role of DAZ in human spermatogenesis is under analysis. Oct4, a nuclear transcription factor, also appears to be critical for the origin of PGCs since it is expressed in cell lineages that give rise to PGCs as well as in PGCs and oocytes but not in sperm once they are in the testes. How these different proteins interact still remains to be revealed.

The following figure summarizes what we know about the origin and formation of the human sex cells.



Clearly, we could spend many lectures on this topic for there is much more to be known about molecular determinants and their functions in germ plasm formation and gametogenesis. The point to be made here is that current molecular methods, coupled with traditional approaches are beginning to shed light on a problem that is fundamental to life and that has concerned scientists for over 100 years.

Final Comments

Our understanding of human development has come from the extensive knowledge gained from pure research on lower animals. Such past and present research continues to guide ongoing research in human embryology and development. Early during development, the fate of the primordial germ cells is determined by endogenous factors (determinants) in lower animals. As expected, the determinants have been found to be genetically controlled. Specific mRNAs appear to be the cytoplasmic determinants that underlie the formation of germ cells. Some likely candidates have been identified and soon the whole molecular and cellular story of germ cell determination will be revealed. Humans share similarities in some of the genes that control germ cell formation. After the PGCs have migrated into the genital ridges, external factors (including hormones) will now influence their further development. Cells that get lost, don't get influenced by a normal set of factors and, as a result, in some instances can form cancerous teratomas. In the female, the number of germ cells increases by mitosis which then stops so that only a limited number of eggs are possible. In fact, as we will shortly see, millions of potential eggs will die along the way. In the male, mitosis and meiosis continue throughout life so a continual supply of sperm is available. The stage is now set for the formation of eggs and sperm. The next lecture examines oogenesis.

References

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