Chapter 15
Germ, Somatic and Stem Cells

A **mutation** in one of your liver cells can never be passed on to your children because the fusion of one sperm cell and one egg cell represents the only genetic link between the bodies of parents and the body of their child and the cells destined to produce sperm and eggs are set aside very early in embryonic life. E.g. By the 15th week of gestation, the human female foetus has already set aside each and every cell that may someday develop into a mature egg. (In fact, each of these cells has already entered its final meiotic division!). The different fates of the germline and somatic cells are controlled by certain proteins that are retained in the germline but destroyed in the somatic cells (by ubiquination).

15.1. Weismann's Theory of the continuity of the germplasm

Over 100 years ago, the German biologist **Weismann** recognized that **animals** are made up of body cells (**somaplasm**), which contain gamete-producing cells (**germplasm**). At each generation, the embryo that develops from the zygote not only sets aside some germplasm for the next generation but also produces the cells that will develop into the body, the **soma**, of the organism.

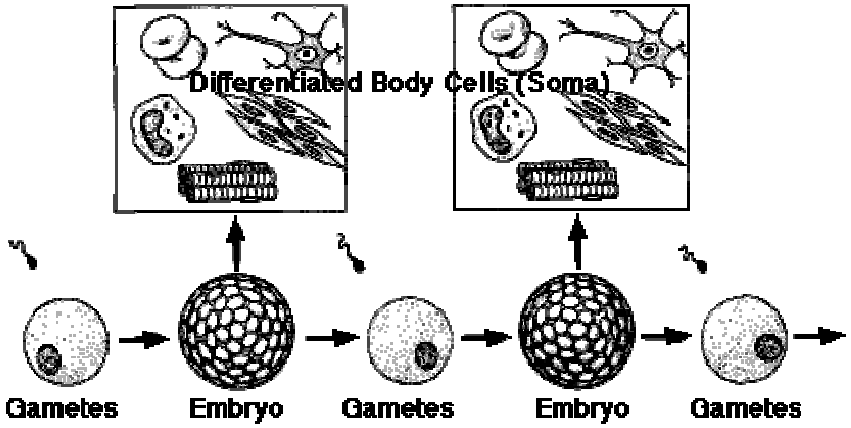

Fig.15.1a. Somaplasm and Germplasm

Today we know that only the germplasm — the gametes and the cells that form them — continue to express high levels of the enzyme **telomerase**. These cells are able to maintain the length of their chromosomes forever and are immortal. The cells of the somaplasm, in contrast, stop producing telomerase, lose a portion of their chromosome tips at each mitosis, and eventually die. In Weismann's view, the somaplasm simply provides the housing for the germplasm, seeing to it that the germplasm is protected, nourished, and conveyed to the germplasm of the opposite sex to create the next generation. The old riddle about which came first, the chicken or the egg, would have been no puzzle to Weismann. In his view, the chicken is simply one egg's device for laying another egg. Weismann also understood the implications of his theory for **aging**. Once the opportunity to pass germplasm on has passed, there is no need to maintain the

integrity of the somaplasm ("disposable soma"); hence the decline in body function with aging .

The mechanisms in insects and mammals are quite different. In insects in appears to be controlled by the special quality of the cytoplasm deposited by the mother at one end of the egg. At the 4th mitotic division in the gall midge (an insect) egg, 2 of the 16 nuclei become pinched off in a small amount of cytoplasm at one end of the egg. At the 5th mitosis, these two nuclei divide normally, producing daughter cells with the full complement of chromosomes (2**n** = 40) of the species. But not so for the other nuclei. When each of these reaches anaphase, only 8 of their 40 chromosomes (dyads) separate and move to opposite ends of the spindle. The remaining 32 chromosomes stay at the equator and eventually disintegrate. The descendants of the two "normal" nuclei ultimately differentiate to form sperm or eggs, i.e., the germline. The descendants of the rest of the nuclei, those with the sharply-reduced chromosome number, go on to form all the other tissues of the insect body, i.e., the soma.

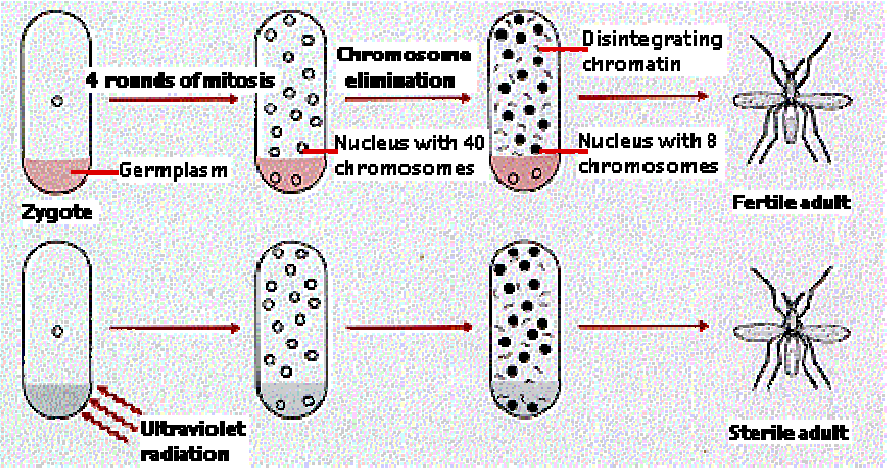

Fig.15.1b. Weismann theory for aging

The top row of this figure shows normal development in the gall midge. The gametes are descended from the two nuclei, each with a full diploid set of 40 chromosomes, that were partitioned off in a mass of special cytoplasm (here called "germplasm"). The remaining nuclei lose 32 chromosomes before going on to form the rest of the insect body (the somaplasm). The bottom row shows that destruction of the germplasm causes the nuclei that move there to undergo chromosome elimination also. The animal that develops is sterile but otherwise normal. In mammals (or at least in mice) the decision to become germ cells is **not** intrinsic but is the result of **cell-cell Signalling** during early embryonic development. The setting aside of the future germ cells begins at the start of **gastrulation** (6 days after fertilization in the mouse) as the mesoderm is forming. These are three steps:

1. In Step 1, Ectoderm cells of the future **extraembryonic membranes** secrete **bone morphogenic proteins,** e.g. Bmp-4, which signal cells in the developing mesoderm to differentiate into cells with the potential to go on to form either mesoderm of the extraembryonic membranes **(amnion** and **allantois**), or primordial germ cells (PGCs)
2. In Step 2, A subset of these cells (at the centre of the cluster) begins to express a transmembrane protein encoded by the gene *fragilis*; a DNA-binding protein **(transcription factor**?) known as stella (also called Pgc7). (Stella/Pgc7 is also

present in the zygote and is found in **embryonic stem cells.**) repress genes (e.g., **homeobox genes** like *Hoxb-1*) needed for development of the somatic tissues of the embryo and extraembryonic membranes.

3. In Step 3, The PGCs migrate into the part of the developing embryo that will go on to form the gonads (ovaries or testes).

15.2. Exceptions to Weismann's Theory

The distinction between germline and soma exists **only in animals**. In **plants**, cells destined to become gametes do arise from somatic tissues. In the flowering plants (**angiosperms**), for example, certain signals cause **meristems** that had been making stem tissue to become converted into flower buds which go on to make the gametes.

In **microorganisms**, all life's functions are embodied in a single cell. (However, some unicellular organisms, like the **ciliated protozoan** *Tetrahymena thermophila*, have a complete genome in their **micronuclei**, which are passed on to the next generation, as well as genes in a **macronucleus**, which is not. Thus, even here, there is the equivalent of a distinction between germline and soma.)

15.3. Somatic vs. Germline Mutations

The significance of mutations is profoundly influenced by the distinction between germline and soma. Mutations that occur in a **somatic cell**, in the bone marrow or liver for example, may damage the cell, make the cell cancerous or kill the cell

Whatever the effect, the ultimate fate of that **somatic mutation** is to disappear when the cell in which it occurred, or its owner, dies. **Germline mutations**, in contrast, will be found in every cell descended from the zygote to which that mutant gamete contributed. If an adult is successfully produced, every one of its cells will contain the mutation. Included among these will be the next generation of gametes, so if the owner is able to become a parent, that mutation will pass down to yet another generation. E.g. More than 8000 people living in South Africa today carry a gene for the metabolic disease called **porphyria**. Every one of them has acquired their gene through a chain of ancestors leading back to a single couple: **Ariaantje Jacobs** and **Gerrit Jansz**. This woman and man emigrated from **Holland** to **South Africa** late in the **seventeenth century** and one or the other of them passed the gene — **through the germline** — on to their descendants. Fortunately, the ailment is usually mild (unless the person is given a **barbiturate sedative**, which triggers a violent reaction).

15.4. Stem Cells

Stem cells are cells that divide by mitosis to form either two stem cells, thus increasing the size of the stem cell "pool", or one daughter that goes on to differentiate, and one daughter that retains its stem-cell properties. How the choice is made is still unknown. However, several genes have been found whose activity prevents a daughter cell from differentiating. Several adjectives are used to describe the developmental potential of stem cells; that is, the number of different kinds of differentiated cell that they can become.

(1) Totipotent Cells

In mammals, totipotent cells have the potential to become any type in the adult body; any cell of the **extraembryonic membranes** (e.g., placenta). The only totipotent cells are the **fertilized egg** and the first 4 or so cells produced by its **cleavage** (as shown by the ability of mammals to produce identical twins, triplets, etc.). In mammals, the expression totipotent **stem** cells is a misnomer — totipotent cells cannot make more of themselves.

(b) Pluripotent Stem Cells

These are true stem cells, with the potential to make any differentiated cell in the body (but probably not all those of the extraembryonic membranes, which are derived from the trophoblast). Three types of pluripotent stem cells have been found

(c) Embryonic Stem (ES) Cells

These can be isolated from the **inner cell mass** (ICM) of the blastocyst — the stage of embryonic development when implantation occurs. For humans, excess embryos produced during **in vitro fertilization** (IVF) procedures are used. Harvesting ES cells from human blastocysts is controversial because it destroys the embryo, which could have been implanted to produce another baby (but often was simply going to be discarded).

(d) Embryonic Germ (EG) Cells

These can be isolated from the precursor to the gonads in aborted foetuses. **Embryonic Carcinoma (EC) Cells.** These can be isolated from teratocarcinomas, a tumour that occasionally occurs in a gonad of a fetus. Unlike the other two, they are usually **aneuploid**. All three of these types of pluripotent stem cells can only be isolated from embryonic or fetal tissue; can be grown in culture, but only with special methods to prevent them from differentiating.

(e) Multipotent Stem Cells

These are true stem cells but can only differentiate into a limited number of types. For example, the bone marrow contains multipotent stem cells that give rise to all the cells of the blood but not to other types of cells. Multipotent stem cells are found in adult animals; perhaps most organs in the body (e.g., brain, liver) contain them where they can replace dead or damaged cells. These **adult stem cells** may also be the cells that — when one accumulates sufficient mutations — produce a clone of **cancer cells**.

15.4 Using Stem Cells for Human Therapy

(a) The Dream

Many medical problems arise from damage to differentiated cells. Examples:

1. **Insulin-dependent diabetes mellitus** (IDDM) where the **beta cells of the pancreas** have been destroyed by an **autoimmune attack;**
2. **Parkinson's disease;** where **dopamine-secreting cells** of the brain have been destroyed;
3. Spinal cord injuries leading to paralysis of the skeletal muscles.
4. Ischemic stroke where a blood clot in the brain has caused neurons to die from oxygen starvation;

5. **Multiple sclerosis** with its loss of myelin sheaths around axons.
6. Blindness caused by damage to the **cornea.**

The great developmental potential of stem cells has created intense research into enlisting them to aid in replacing the lost cells of such disorders. While some success has been achieved with laboratory animals, not much has yet been achieved with humans. One exception: culturing human epithelial stem cells and using their differentiated progeny to replace a damaged cornea. This works best when the stem cells are from the patient (e.g. from the other eye). Corneal cells from another person (an allograft) are always at risk of rejection by the recipient's immune system.

b) The Problems

So one major problem that must be solved before human stem cell therapy becomes a reality is the threat of rejection of the transplanted cells by the host's immune system (if the stem cells are allografts; that is, come from a genetically-different individual).

(c) The Solution

One way to avoid the problem of rejection is to use stem cells that are genetically identical to the host. This is already possible in the rare situations when the patient has healthy stem cells in an undamaged part of the body (like the stem cells being used to replace damaged corneas). But even where no "autologous" stems cells are available, there may be a solution: using **somatic-cell nuclear transfer** (but with no goal of attempting to implant the resulting blastocyst in a uterus). In this technique, a human egg has its own nucleus removed and replaced by a nucleus taken from a somatic (e.g., skin) cell of the patient. The now-diploid egg is allowed to develop in culture to the blastocyst stage when embryonic stem cells can be harvested and grown up in culture. When they have acquired the desired properties, they can be implanted in the patient with no fear of rejection. While an exciting prospect, there are still problems with the method that must be solved.

(i) Imprinted Genes

Sperm and eggs each contain certain genes that carry an "imprint" identifying them later in the fertilized egg as being derived from the father or mother respectively. Creating an egg with a nucleus taken from an adult cell may not allow a proper pattern of imprinting to be established. When the diploid adult nucleus is inserted into the enucleated egg (at least those of sheep and mice), the new nucleus becomes "reprogrammed". What reprogramming actually means still must be learned, but perhaps it involves the proper **methylation and demethylation** of imprinted genes. For example, the **inactive X chromosome** in adult female cells must be reactivated in the egg, and this actually seems to happen.

(ii) Aneuploidy

In primates (in contrast to sheep, cattle, and mice), the process of removing the resident nucleus causes molecules associated with the **centrosome** to be lost as well. Although injecting a donor nucleus allows mitosis to begin, spindle formation may be disrupted, and the resulting cells fail to get the correct complement of chromosomes (**aneuploidy**).

(iii) Somatic Mutations

This procedure also raises the spectre of amplifying the effect(s) of somatic mutations. In other words, mutations that might be well-tolerated in a single somatic cell of the adult

(used to provide the nucleus) might well turn out to be quite harmful when they become replicated in a clone of cells injected later into the patient.

(iv) Political Controversy

The goal of this procedure (which is often called "**therapeutic cloning**" even though no new individual is produced) is to culture a **blastocyst** that can serve as a source of **ES cells**. But that same blastocyst could theoretically be implanted in a human uterus and develop into a baby that was genetically identical to the donor of the nucleus. In this way, a human would be cloned. And in fact, Dolly and other animals are now routinely cloned this way. The spectre of this is so abhorrent to many that they would like to see the procedure banned despite its promise for helping humans. In fact, many are so strongly opposed to using human blastocysts — even when produced by nuclear transfer — that they would like to limit stem cell research to **adult stem cells** (even though these are only multipotent).

Two possible solutions (both so far demonstrated only in mice): ES cells can be derived from a **single cell** removed from an 8-cell **morula**. We know that, in humans, removing a single cell from the morula does not destroy it — the remaining cells can develop into a blastocyst, implant, and develop into a healthy baby. In altered nuclear transfer (**ANT**) — a modified version of SCNT (**somatic-cell nuclear transfer**) — a gene necessary for later implantation (*Cdx2* — endocoding a **homeobox transcription factor**) is turned off (by **RNA interference**) in the donor nucleus before the nucleus is inserted into the egg. The blastocyst that develops has a defective **trophoblast** that cannot implant in a uterus; but the cells of the **inner cell mass** are still capable of developing into cultures of ES cells. (The gene encoding the interfering RNA can then be removed using the Cre/*loxP* technique.). If these procedures work in humans, neither would involve the destruction of a potential human life.