Is the Germline Immortal and Continuous? A Discussion in Light of iPSCs and Germline Regeneration

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ABSTRACT

The germline gives rise to gametes, is the hereditary cell lineage, and is often called immortal and continuous. However, what exactly is immortal and continuous about the germline has recently come under scrutiny. The notion of an immortal and continuous germline has been around for over 130 years, and has led to the concept of a barrier between the germline and soma (the "Weismann barrier"). One repercussion of such a barrier is the understanding that when the germline is lost, soma cannot replace it, rendering the organism infertile. Recent research on induced pluripotent stem cells (iPSCs) and germline regeneration raise questions about the impermeability of the Weismann barrier and the designation of the germline as immortal and continuous. How we conceive of the germline and its immortality shapes what we perceive to be possible in animal biology, such as whether somatic cells contribute to the germline in some metazoans during normal development or regeneration. We argue that reassessing the universality of germline immortality and continuity across all metazoans leads to big and exciting open questions about the germ-soma cell distinction, cell reprogramming, germline editing, and even evolution.

1.0 Introduction

The germline is the lineage of reproductive cells that includes gametes and their precursors, including primordial germ cells and germline stem cells. Because the germline gives rise to the gametes, it is the hereditary cell lineage, and is ultimately responsible for all cells in an organism's body, including the next generation of the germline, stem cells, and other somatic cells. The germline is often called immortal because of its role in heredity and the propagation of new generations; however, what, exactly, is immortal about the germline has recently come under scrutiny (Hayflick, 2019; Yamashita, 2019). In this essay we examine what germline immortality means and raise challenges to the notion of this immortality from recent work on induced pluripotent stem cells (iPSCs) and germline regeneration.

While our discussion of germline immortality relies on semantics, our arguments should not be considered as mere nitpicking. Understanding how we conceive of the germline and its immortality has real world consequences, notably, the possibility that somatic mutations can get passed on to new generations when the germline "dies" leads to big, open questions about cell reprogramming, germline editing, and evolution.

2.0 Immortality Means Continuity

Explicit definitions of immortality in relation to the germline are rarely given. However, immortality seems to equate with continuity across (and presumably within) generations. Because of this equivalence, we briefly discuss what continuity could mean with regards to the germline and point out problems associated with each form of continuity. From our review of the literature, we suggest that there are three forms of continuity linked with immortality: 1) continuity of material, 2) continuity of information, and 3) continuity of cell lineage.

2.1 Immortality as Continuity of Material

There are two candidates for the germline material that could ensure continuity within and between generations: cells and germ plasm (DNA is discussed in the following section). The continuity of each of these materials faces serious challenges. Cells, for instance, experience a quantitative reduction in number at fertilization (i.e. two germ cells unite to form a single zygote), their molecular constituents are constantly turned over, and individual cells are not immortal (Hayflick, 2019). Germ plasm, on the other hand, plays a role in specifying the germline (see Section 3.0). However, germ plasm has not been found in all organisms, nor is there evidence that the exact material itself is continuous throughout an organism's germline or across generations.

2.2 Immortality as Continuity of Information

DNA contains the information for heredity and development. However, DNA undergoes changes during meiosis: the chromosomes within what will become the gametes undergo genetic recombination, ensuring that the information contained in the DNA of gametes (and resulting progeny) is not a perfect copy of the parent (Wylie, 1999). Additionally, during fertilization, both sperm and egg contribute to the zygote, creating a version of the genome that did not exist in either parent (Fig. 1A). Thus, there is not perfect fidelity of information between generations. To what degree, then, is fidelity of information between generations necessary in order for us to call this information continuous?

If we set aside the issue of information fidelity and say that DNA is the immortal component of the germline, then germline immortality is equivalent to the flow of genetic information between generations (heredity). In this line of thinking, a distinct and continuous lineage of cells (germline) that carries the DNA becomes irrelevant because the transfer of DNA between generations, and not a specific cell lineage, is all that matters for heredity. Thus, we question the usefulness of thinking of germline immortality as continuity of information because it is no different from the theory of heredity.

2.3 Immortality as Continuity of the Cell Lineage

The final and most prevalent form of continuity within the literature is cell lineage continuity, or the idea that there is "preservation of a continuous germ lineage over successive generations" (Gartner *et al.*, 2018). In what way a germline cell lineage can remain continuous from one generation to the next is far from clear. This lack of clarity concerns two different, albeit related, problems: continuity of a germline cell lineage *between generations* and *within an organism*.

The argument supporting continuity of cell lineage between generations often runs along the following lines: germline continuity exists because the germline connects generations going back to the evolutionary origin of germline. This argument calls to mind Rudolph Virchow's 1855 dictum: *Omnis cellula e cellula* ("All cells [come] from cells") and seems to state nothing more than one of the main tenets of cell theory. Therefore, relying on it does not tell us anything more about the germline than that it conforms to cell theory. Second, creating the zygote through sexual reproduction requires a quantitative reduction in the germline cells (Fig. 1A).

The second problem with conceptualizing germline cell continuity as the basis for immortality is understanding what it means for the germline to be continuous within an organism. As we show in the following sections, assuming this form of continuity is particularly problematic, especially in light of recent research on iPSCs and germline regeneration.

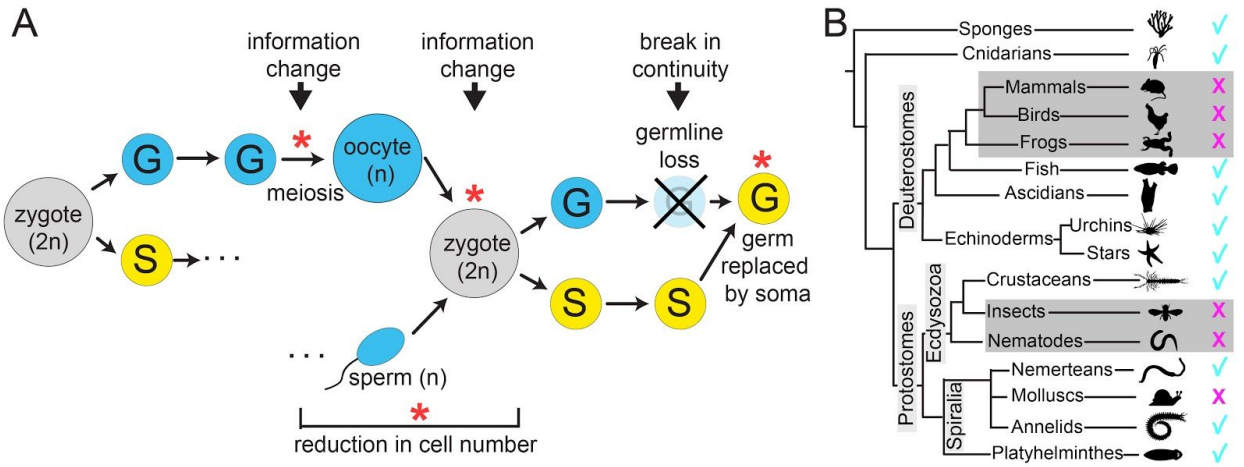

Figure 1. A) Which component of the germline is immortal and continuous? Stars: points of change. Cells originating from germline (G) in blue, from soma (S) in yellow. **B)** Distribution of germline regeneration across animal phyla. Gray indicates the phylogenetic location of traditional model organisms which cannot regenerate the germline *in vivo* (indicated by "X"). Check marks indicate clades in which there is evidence for germline regeneration.

3.0 From Continuity of Cell Lineage to Germline Specification and the Weismann Barrier

Let's pick up the question that we left at the end of the previous section: Is there a continuous germline cell lineage within organisms? In order to address this question, we need to look at the ways in which the germline is specified during development. There are two modes of germline specification in metazoans: maternal inheritance and induction. Maternal inheritance is considered continuous because it relies on the direct contribution of parental material (the germ plasm) to the newly-formed embryo to specify the germline early on in development (Extavour and Akam, 2003; Trcek and Lehmann, 2019). Induction is typically considered discontinuous (Extavour, 2007) because the germline is induced to form at a later stage of development. However, there is some debate about whether induction is truly discontinuous because somatic cells do not become a part of the germline once the germline has formed (Lawson *et al.*, 1999). While we could reason by looking at modes of germline specification that there are many instances in which the germline is discontinuous, for argument's sake, we will adopt a looser definition of cell lineage continuity for the moment and say that germline cell lineage is continuous within an organism if, once specified, it does not incorporate any somatic cells.

The question of whether or not an organism incorporates soma into a designated germline is as old as asking about the role of the germline in heredity. August Weismann, one of the earliest and most influential germline researchers, conceived of a "barrier" between the germ and soma, such that the germline can give rise to soma, but soma cannot give rise to germ (note that this aligns exactly with our definition of germline cell lineage continuity above). We now call this the Weismann barrier, and it is largely considered inviolable in part because it maintains a continuous germline which does not allow for somatic mutations to pass on to future generations.

The Weismann barrier is considered fundamental to biology. However, recent research on stem cells and germline regeneration challenge its universal applicability by raising serious questions about germline cell lineage continuity (and immortality).

4.0 Violating the Weismann Barrier and Breaking Germline Cell Lineage Continuity

One of the major implications of an inviolable Weismann barrier is that a complete loss of the germline should lead to sterility because the germline cannot regenerate (i.e. germline that is lost cannot be replaced by soma). This view has been tested and upheld within research on traditional animal models such as *Drosophila*, *C. elegans*, and *Xenopus* (Everett, 1943; Züst and Dixon, 1975; Sulston *et al.*, 1983; Barnes *et al.*, 2006). However, the generalizability of these observations from model organisms and the concept of germline immortality versus somatic

mortality has been challenged by many others in the past century (Heys, 1931; Bounoure, 1940; Berrill and Liu, 1948; Nieuwkoop and Sutasurya, 1979; Buss, 1983; Extavour, 2007; Solana, 2013). Indeed, research conducted over the past few decades on iPSCs and non-traditional model organisms leads us seriously to question whether the Weismann barrier is a valid universal within metazoans.

Research largely conducted on mice has led to the understanding that lost or artificially removed germ cells can be regenerated using iPSCs. These stem cells, which originate from harvested somatic cells (such as fibroblasts in the adult mouse tail tip), can give rise to mature gametes which produce fertile offspring in previously infertile mouse strains (Hayashi *et al.*, 2011). While using iPSCs to replace missing germline is not an instance of *in vivo* germline regeneration, this work suggests that somatic cells have the potential to be reprogrammed into germline and that the Weismann barrier can be broken under artificial conditions.

Second, observations and experiments in non-traditional model organisms have shown that many metazoans readily regenerate their germline over a large span of life stages and do so in a variety of ways, including by converting somatic lineages to germline (Takamura *et al.*, 2002; Wang *et al.*, 2007; Yajima and Wessel, 2011; Leclère *et al.*, 2012; Özpolat *et al.*, 2016; Yoshida *et al.*, 2017; Dannenberg and Seaver, 2018). The following cases highlight the variety of ways in which metazoans can regenerate their germlines *in vivo*.

Flatworms have stem cells (neoblasts) that give rise to both germ and soma (Morgan, 1902; Wang *et al.*, 2007). Whether there is a sub-population of neoblasts that are tasked with regenerating only the germline remains to be determined. Adult segmented worms can regenerate germ cells when regenerating half of their body axis (Tadokoro *et al.*, 2006; Giani *et al.*, 2011; Özpolat and Bely, 2016; Özpolat *et al.*, 2016); and annelid embryos develop into fertile adults following germline ablation (Dannenberg and Seaver, 2018). When the larval tail containing the primordial germ cells of the tunicate *Ciona intestinalis* is removed, the regenerating organism readily transitions somatic lineages into germline, resulting in adults that give rise to fertile progeny (Takamura *et al.*, 2002; Yoshida *et al.*, 2017).

This research on iPSCs indicates that the Weismann barrier is permeable; while the research on germline regeneration indicates that the Weismann barrier, and thus germline cell lineage continuity, is frequently broken within metazoans. And, the fact that somatic cells can redifferentiate into germline cells indicates whatever mechanisms specify the germline (1) exist within at least some somatic cells, and (2) can be reinitiated under certain circumstances. The consequences of this are discussed below. Meanwhile, questions surrounding the extent to which metazoans regenerate their germlines, and the cellular sources and molecular mechanisms of germline regeneration are all wide-open areas of inquiry.

5.0 Open Questions & Conclusion

Recent research in stem cells and germline regeneration has undermined some of the principles of an immortal germline, such as the idea that soma cannot convert to germ (e.g. the Weismann barrier). The recently uncovered potential of somatic cells to convert to germline opens up a number of big questions in biology. For instance, is there a selection process among

somatic cells when they redifferentiate into germline during regeneration? And, what are the mechanisms of soma-to-germ reprogramming? Answering these questions will significantly inform research areas such as cell reprogramming, maintenance of genome integrity, stem cell therapies, and infertility treatments. Furthermore, one of the corollary assumptions of the Weismann barrier is that the inability of soma to convert to germ protects the germline from incorporating somatic mutations. If soma can convert to germ, which appears to be the case in at least some animals and many plants, the evolutionary and population genetics consequences need to be considered. For example, we may need to rethink some of our reasoning about germline editing, which relies on the assumption that soma cannot contribute to the germline.

The idea that germline can regenerate comes as a surprise to many even though evidence suggests it occurs widely throughout metazoans. We contend that this is due, in part, to the longstanding assumption of germline continuity and immortality. Afterall, if we take the continuity of the germline (especially cell lineage continuity) as a given, then there is no impetus to look for cases in which this continuity is broken. With this essay, our hope is to shed light on problems and questions that have been obscured by this rhetoric. The Weismann barrier and notions of immortality and continuity need not be discarded, they must simply be questioned, and their usefulness determined on a case-by-case basis throughout metazoans.

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