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The wisdom of Weismann:

Epigenetic erasure mechanisms and germ line immortality

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As first proposed by August Weismann in 1893, the germline is both the repository and the source of heritable information that is passed between generations. In the ensuing 20th century, it was recognized that DNA is one component of information that is passed between generations. Towards the end of the 20th and now into the 21st century, it became clear that information surrounding the DNA sequence—or epigenetic information—is another component. Furthermore, as predicted by Weismann, the germline is becoming recognized as not only the guardian but perhaps also the editor of the information that is transferred. Indeed, the more we learn about the continuity or “immortality” of the germline, and the molecular nature of the *germplasm* described by Weismann, the more we have come to appreciate the insight of this amazingly creative thinker and founding father of developmental biology.

Recognition of the importance of transgenerational epigenetic information has not come from an understanding of the information content itself—of which we still have little understanding—but instead from the discovery of processes that are important for its careful *erasure* in the embryo. For example, there are successive waves of genome-wide DNA methylation removal that occur in the mammalian zygote accompanied by large scale changes in heterochromatin structure (reviewed in ref. 1). The importance of these events can be inferred from early cloning experiments, in which terminally differentiated nuclei regain pluripotency, with increasing efficiency upon repeated exposure to oocyte cytoplasm, the *germplasm* to which Weismann referred. This “reprogramming” process likely involves DNA demethylase and histone modifying activities that have only recently been identified and whose characterization is incomplete. Furthermore, the excitement caused by the recent development of induced pluripotent stem cells (iPS cells), in which forced expression of a remarkably minimal set of factors can restore pluripotency in adult somatic cells, is tempered by the inefficiency of the technique and the lack of mechanistic information about the processes that are induced.

At least two important concepts can be appreciated after several decades of cloning experiments: (a) the reversal of the differentiated state involves a reversal of the epigenetic information imposed by differentiation, and (b) the *germplasm* carries the tools to perform this reversal, and normally accomplishes this feat at each generation. The question remains: what are the endogenous targets of the epigenetic reprogramming mechanisms in the

zygote? During somatic cell nuclear reprogramming, the epigenetic landscape that maintains the differentiation-specific phenotype has to be erased and reprogrammed to the pluripotent ground state. It is thus reasonable to assume that, at each generation, epigenetic information acquired during development of the highly differentiated gametes would also need to be reprogrammed by this process. There has been little direct evidence to date that this is the case; however, we propose that the recent results of experiments our lab performed in the nematode *C. elegans* may provide such evidence.²

There is no DNA methylation in *C. elegans*, and thus all epigenetic information is presumably encoded in histone modifications and their consequences. One particular histone modification, histone H3 di-methylated on lysine 4 (H3K4me2) is dramatically erased from the genome during primordial germ cell (PGC) specification.³ This process may be analogous to epigenetic erasure mechanisms that are observed in PGCs of other organisms.^{4,5} Histone demethylases, such as the mammalian co-repressor component Lsd1, have been shown to actively remove this mark in other systems.⁶ We therefore tested whether worm homologs of Lsd1 were involved in the H3K4me2 removal in the PGCs. Mutants in all three homologs, alone and in all combinations, did not show a defect in this process; a result that was initially disappointing. However, it was noticed that as mutant lines were maintained, they produced fewer progeny, and that the progeny were often sterile. Careful generational analysis showed that continued passage of strains defective in one particular homolog, *spr-5*, yielded sterile animals with increasing frequency in successive generations; the hallmarks of a “germline mortality” phenotype. Furthermore, there was no evidence of accumulating genetic defects. Instead, the population seemed to be accumulating *epigenetic* defects, since the phenotypes could be reversed by transient exposure to SPR-5 demethylase activity.

Microarray experiments that compared expression profiles between multiple generations showed striking and coordinated expression changes in one particular class of genes—those expressed in spermatogenesis. The expression of these genes climbed in early generations until peaking near the generations in which fertility plummeted—at which point the expression of these genes also fell. Importantly, the level of H3K4me2 in the promoter chromatin of these genes continued to increase despite the loss of expression. This indicated that there was a persistence of aberrant epigenetic accumulation in successive generations without increased transcription. Furthermore, in these later stages there was an increased failure to efficiently erase H3K4me2 from PGC chromatin, which suggested that the SPR-5-independent erasure mechanism was being overwhelmed. We concluded from these studies that histone demethylases are required to remove epigenetic information acquired in the parental germline, and that defects in this erasure lead to its persistence and accumulation in successive generations. This ultimately causes a failure of proper gametogenesis—and an end to germline immortality. Over 100 years ago August Weismann proposed that germplasm endows a special property upon the germline—the ability to differentiate into gametes, yet restore totipotency following fertilization. Our studies in *C. elegans* suggest that H3K4me2 demethylation by the Lsd1 demethylase SPR-5 may be a part of this special property.

Important questions remain: Where in the “germline cycle” is the histone demethylase activity required? Antibodies to SPR-5 recognize the protein in the adult germ cells of both sexes as well as in the early embryo, so it could be functional in either or both stages. Why does the defect take multiple generations to unfold? One possibility is that the H3K4me2 erasure mechanisms in the PGCs provide an imperfect back-up erasure system, such that successive generations come with a stochastically increasing level of H3K4me2 to be erased. Interestingly, in *Drosophila* PGC-specific H3K4me2 erasure doesn't appear to occur, and mutants in the fly Lsd1 ortholog are sterile in the first generation of homozygosity.^{7,8} Another important question concerns the targeting of erasure mechanisms; how does any mechanism determine what information is to be erased and what is to be maintained?

It is interesting to note that a number of studies showing transgenerational epigenetic phenomena in mammals point out that the critical window of the initiating event falls within the period of fetal germline development (reviewed in refs. 9 and 10). This indicates that the consequences of epigenetic events occurring in the germline, if unchecked, have the capacity to unfold for many generations. Incredibly, this is consistent with Weismann's original hypothesis of over a century ago that modes of heredity are limited to the germline, and thus events in the germline can have consequences for multiple generations and impact the evolution of species.

References

1. Reik W. *Nature*. 2007; 447:425–432. [PubMed: 17522676]
2. Katz DJ, et al. *Cell*. 2009; 137:308–320. [PubMed: 19379696]
3. Schaner CE, et al. *Dev Cell*. 2003; 5:747–757. [PubMed: 14602075]
4. Seki Y, et al. *Dev Biol*. 2005; 278:440–458. [PubMed: 15680362]
5. Hajkova P, et al. *Nature*. 2008; 452:877–881. [PubMed: 18354397]
6. Shi Y, et al. *Cell*. 2004; 119:941–953. [PubMed: 15620353]
7. Rudolph T, et al. *Mol Cell*. 2007; 26:103–115. [PubMed: 17434130]
8. Di Stefano L, et al. *Curr Biol*. 2007; 17:808–812. [PubMed: 17462898]
9. Roemer I, et al. *Curr Biol*. 1997; 7:277–280. [PubMed: 9094308]
10. Anway MD, et al. *Science*. 2005; 308:1466–1469. [PubMed: 15933200]