# Novel improvement in iPSCs generation protocols through mouse gametogenesis epigenetic erasure mechanisms.

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The main goal of this review is to describe gametogenesis epigenetic erasure mechanisms and to propose candidate molecules which can erase remaining epigenetic marks that enhance re-differentiation of cultured induced pluripotent stem cells (iPSCs) into their tissue of origin. For accomplishing this objective, the most representative epigenetic events occurring during gametogenesis are detailed, because those are responsible for erasing parental epigenetic marks. Moreover, early embryonic development is also studied for describing pluripotency establishment. Finally, different epigenetic regulators from the three studied mechanisms are proposed as candidates to improve iPSCs generation protocols.

## Epigenetic reprogramming

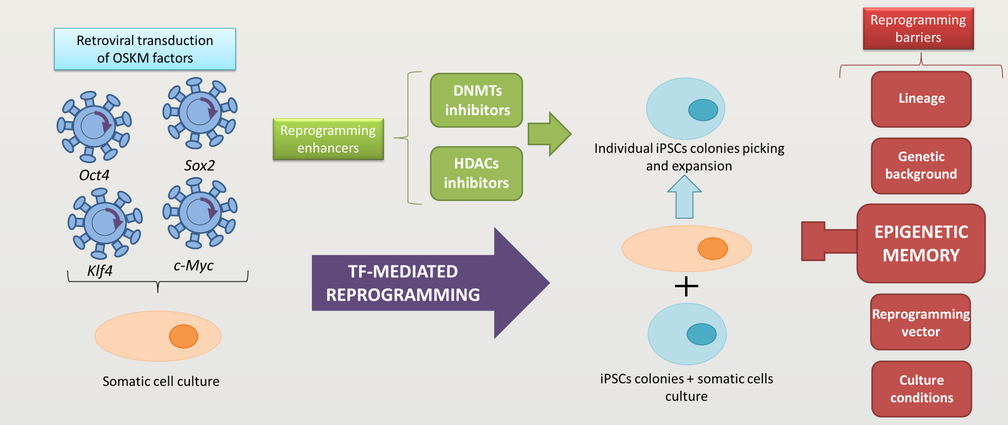


**Figure 1. Transcription factor (TF)-mediated reprogramming as described by Takahashi and Yamanaka in 2006**. Reprogramming has some barriers, although it can be enhanced by DNA methyltransferases (DNMTs) inhibitors and histone deacetylases (HDACs) inhibitors.

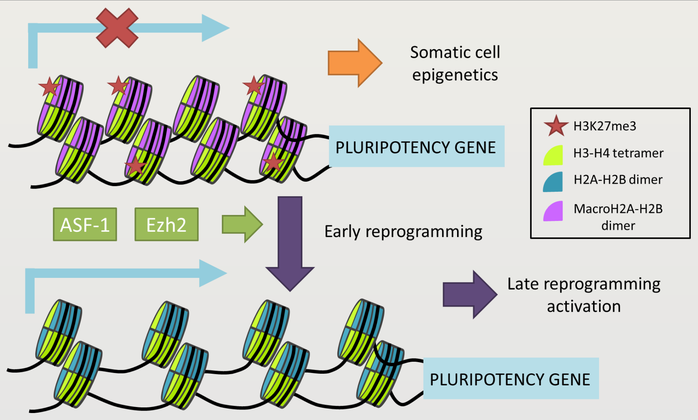


**Figure 2. Epigenetic reprogramming**. During early reprogramming the repressive histone modification H3K27me3 (trimethylation at lysine 27 of histone H3) and macroH2A are lost. ASF-1 histone chaperone and PCR2 (polycomb repressive complex 2) catalytic subunit Ezh2 enhance reprogramming efficiency.

TF-mediated reprogramming is thought to be the best strategy to generate patient specific iPSCs for disease modelling and cellular replacement therapies, among other applications. However, it is associated with incomplete epigenetic reprogramming leading to re-differentiation of cultured iPSCs into their tissue of origin (Figure 1). Epigenetic reprogramming is the removal of existing epigenetic information and the establishment of a new one (Figure 2). When this process fails, remaining epigenetic marks lead to epigenetic memory. Even though this barrier can be palliated by the addition of some drugs, which stimulate an optimal chromatin configuration, this is not enough to overcome epigenetic memory. For this reason, epigenetic events during gametogenesis and early embryonic development are reviewed.

## Cell memory erasure mechanisms

DNA demethylation after midgestation erases parental information, preparing cells for sex-specific *de novo* methylation during gametogenesis. Demethylation can be achieved actively (Figure 3) or passively (Figure 4).

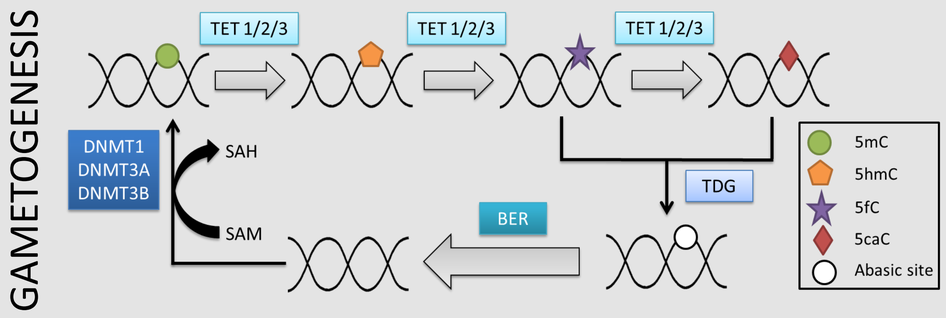


**Figure 3. Active DNA demethylation by a TET/TDG/BER-dependent pathway**. 5-methylcytosine (5mC) is iteratively oxidized by TET enzymes producing 5-hidroximethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxycytosine (5caC). 5fC and 5caC can be excided by TDG and the abasic site is repaired by BER proteins.

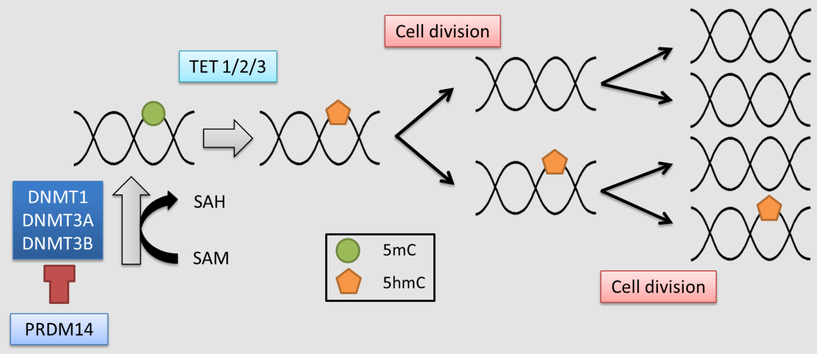


**Figure 4. Passive DNA demethylation**. DNA methylation is lost on the DNA strand opposite to 5hmC. Newly replicated DNA dilutes 5hmC through cell divisions.

There are two waves of reprogramming in mammalian development. The first one takes place during primordial germ cells migration and gonad formation, allowing existing epigenetics removal and establishing a different epigenome. The second, occurs shortly after fertilisation in early preimplantation embryo (Figure 7). During the late stages of gametogenesis, oocyte (Figure 5) and sperm (Figure 6) undergo extensive chromatin remodelling and nuclear condensation, required to silence germline transcriptional programmes and acquire a developmentally compete nuclear state capable of supporting early embryogenesis. After fertilisation DNA methylation levels are globally reduced to their lowest levels in the inner cell mass.

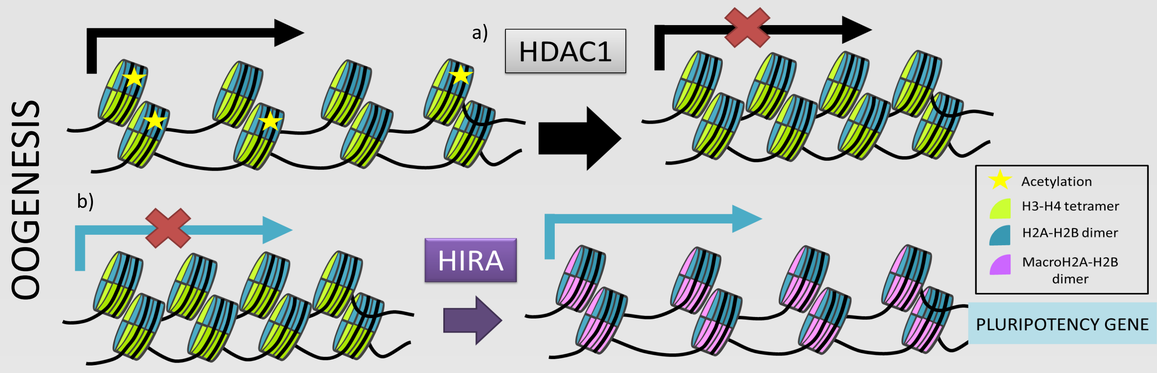


**Figure 5. Oogenesis epigenetic events**. HDAC1 accessibility to chromosomes explains meiosis-specific deacetylation and decreased acetylation levels (Figure 5a). H3.3 histone variant is a crucial maternal factor for reactivating pluripotency genes (Figure 5b).

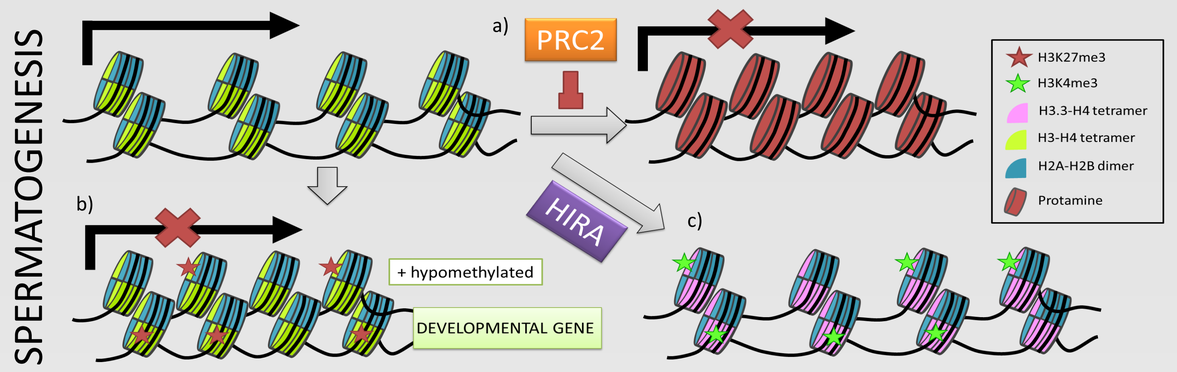


**Figure 6. Spermatogenesis epigenetic events**. Histones are exchanged for protamines, but PCR2 inhibits nucleosome turnover (Figure 6a). Developmental important loci are hypomethylated and retain nucleosomes and H3K27me3 (Figure 6b). Residual nucleosomes basically contain modified H3.3 variant (Figure 6c).

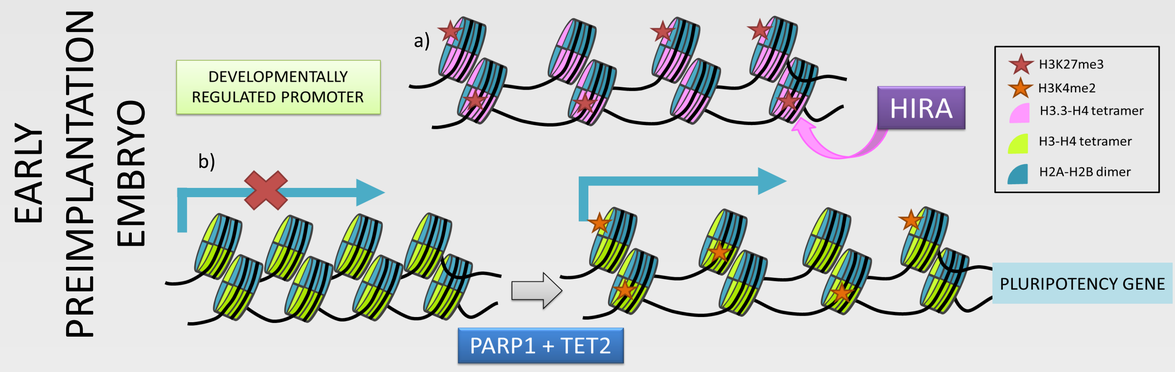


**Figure 7. Early embryonic epigenetic events**. H3K27me3 establishment at developmentally regulated promoters in ESCs require HIRA-mediated H3.3 deposition (Figure 7a). Poly(ADP-ribose) polymerase-1 (PARP1) and TET2 are needed for early histone modification establishment (Figure 7b). PARP1 induction promotes OCT4 accessibility to *Nanog* and *Esrrb* promoters.

## MAIN CONCLUSIONS

This review proposes as good candidates to improve iPSCs generation protocols the following molecules: ASF-1 histone chaperone, Ezh2 subunit, PRDM14, TET enzymes, HIRA histone chaperone, other PRC2 subunits and PARP1. It must be taken into consideration that they cannot be simultaneously induced, as redundancy could overshadow their impact. For this reason, further experiments must be done in order to elucidate which ones are better enhancers of reprogramming.

## Novel improvement in iPSCs generation protocols

The main objective is to ensure proper DNA demethylation and histone modification erasure. Drugs in the medium will facilitate OSKM factors and transduced molecules accessibility to chromatin, which will improve iPSCs generation efficiency by overcoming cell memory. This proposal's main limitation is that it is not taking into consideration non-coding RNAs (ncRNAs), which also have an important role in epigenetic memory.

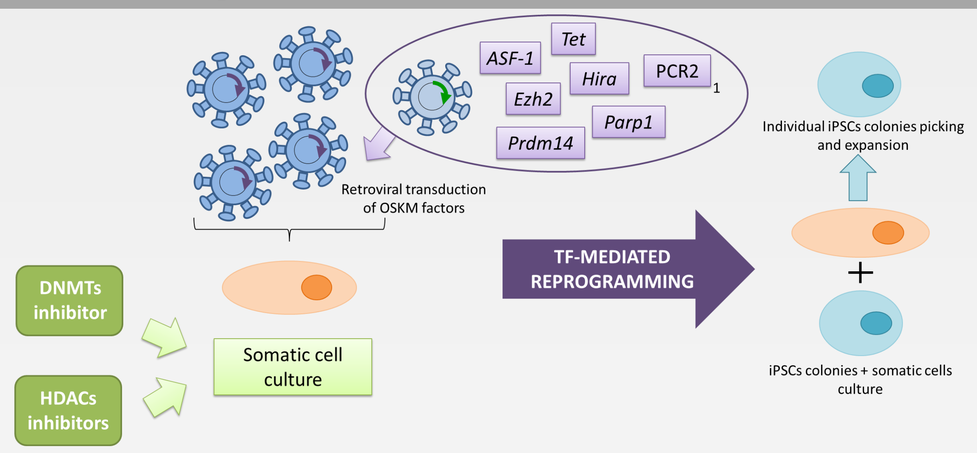


**Figure 8. Proposed experimental mechanism**. OSKM transduction in cells cultured in supplemented medium with DNMTs and HDACs inhibitors. Posteriorly, transduction of one or some of the candidate molecules into early-stage reprogramming cells (purple oval). [1]Different PCR2 subunits apart from Ezh2.

**KEY REFERENCES:**

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