CHAPTER 31

# Genomic Reprogramming

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## 31.1 INTRODUCTION

From the earliest speculation about genomic reprogramming through many subsequent experiments, it has become clear that the maternally inherited factors contained within the oocyte also have the extraordinary property to restore totipotency to a differentiated somatic nucleus when transplanted into it. The components within the oocyte must have the property to alter the somatic nucleus so that it can recapitulate the entire developmental program, and thus give rise to an exact genetic copy or clone of the individual who donated the transplanted nucleus. This transformation of differentiated cell to a totipotent state is probably the most widely understood meaning of genomic reprogramming. However, it is important to note that extensive epigenetic reprogramming of the genome also occurs in the germ line and during early development, which is essential for generating the totipotent zygote, and for creating the pluripotent epiblast cells from which both germ cells and somatic cells are subsequently derived.

Specification of diverse cell types from pluripotent cells is determined by the expression of a precise set of genes, while the rest are repressed. These newly acquired cell fates are propagated by heritable epigenetic mechanisms through modifications of chromatin and by DNA methylation. These epigenetic modifications, although they are heritable, are also reversible and can be erased, which is why it is possible to change the phenotypic characteristics of cells and restore totipotency to somatic nuclei under specific conditions (see Figure 31.1). To understand the mechanisms of reprogramming, it is important to know the nature of chromatin modifications and the mechanisms that can reverse or erase the existing modifications, and also how new modifications are imposed. Because these reprogramming factors normally play a significant role during early development, it is important to determine



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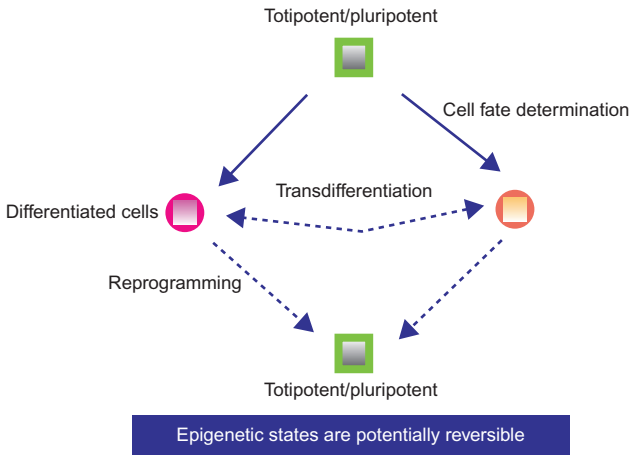


**FIGURE 31.1** Genomic reprogramming involves heritable but reversible epigenetic modifications.

their role in this context, and how these factors act on somatic nuclei during restoration of totipotency or pluripotency.

## 31.2 GENOMIC REPROGRAMMING IN GERM CELLS

Germ cells provide the enduring link between generations, and for this reason this lineage exhibits many unique properties, including the extensive epigenetic reprogramming of the genome prior to gametogenesis. This reprogramming is crucial for generating viable and functional gametes, which in turn generate a totipotent zygote. Primordial germ cells (PGCs) are among the first cells to undergo specification from pluripotent epiblast cells, when the distinction between germ cells and soma is established. PGCs as precursors of sperm and oocyte are highly specialized cells, and are the only cells that can undergo meiosis. However, PGCs retain expression of some markers of pluripotency, such as *Oct4*. It is also possible to derive pluripotent embryonic germ cells (EG) from PGCs. In this context, it is interesting to determine both how PGC specification occurs, and how these cells undergo dedifferentiation to pluripotent EG cells, which may provide some insights into genomic reprogramming.

### 31.2.1 Stem Cell Model for the Specification of Germ Cells in Mammals

There are two key mechanisms for the specification of germ cells. The first involves inheritance of preformed germ plasm, which is found in *Drosophila*

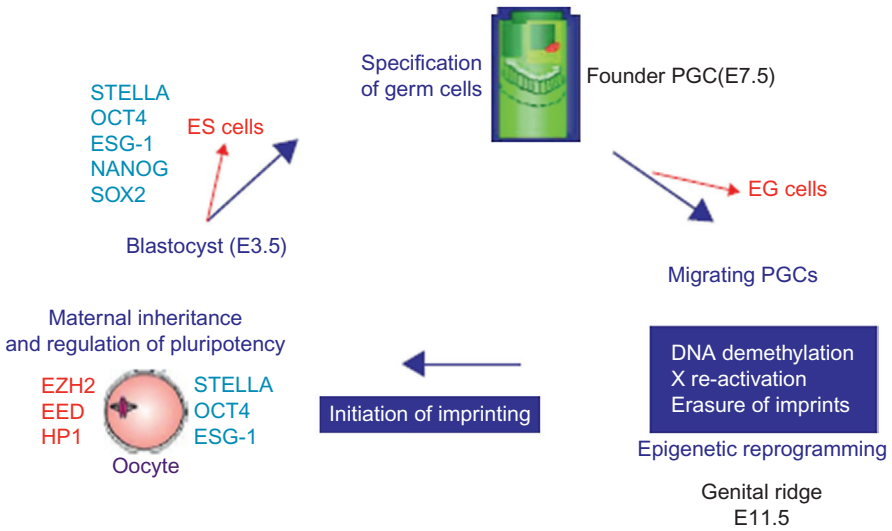


**FIGURE 31.2** **Genomic reprogramming in the oocyte, early embryos, and the germ line.**
The origins of pluripotent embryonic stem cells (ES) and embryonic germ cells (EG) are indicated.

and *C. elegans*. In mammals, germ cell specification occurs according to the stem cell model, where germ cells are derived from pluripotent epiblast cells in response to signaling molecules from the extraembryonic ectoderm. BMP4 and BMP8b are among the key signaling molecules in conferring germ cell competence on pluripotent epiblast cells in mice, starting at E6.5 (Figure 31.2), an event that is detected by the expression of *fragilis*, a transmembrane protein. These germ cell competent cells are initially destined for a mesoderm somatic cell fate, as they show expression of *Brachyury* and some region-specific *Hox* genes as they migrate toward the posterior proximal region. However, at around E7.25, cells that ultimately acquire a germ cell fate switch off the somatic program through repression of a number of genes, which continue to be expressed in the neighboring somatic cells. Cells that acquire germ cell fate continue to show expression of markers of pluripotency, including *Oct4*. A unique marker of germ cells at this time is Stella, which is first detected in the 45–50 founder germ cells at E7.5. Thus, diversification between germ cell and somatic cell fate among neighboring cells occurs over approximately 6–10 hours between E7.25 and E7.5, with the repression of somatic cell programming being one of the major events during germ cell specification.

#### *31.2.1.1 From Stem Cells to Germ Cells*

Recent studies show that it is also possible to derive PGCs and gametes from pluripotent stem cells. In one study, embryonic stem (ES) cells with the

*gcOct4-GFP* reporter, which drives expression specifically in germ cells, were generated. The ES cells containing the reporter were allowed to undergo differentiation when cells with GFP were detected. These cells expressed a variety of germ cell-specific markers. Further cultures following cell sorting eventually produced oocyte-like cells that underwent development to form blastocyst-like structures. The latter shows that mouse ES cells can, under these conditions, differentiate into oocytes and, subsequently, blastocysts. With further detailed characterization of germ cells and gametes, such an *in vitro* system may be useful for investigations concerning specific aspects of germ cell development.

Another study has similarly been carried out to generate spermatogenic cells from pluripotent ES cells. In this case, the endogenous mouse homolog of *Vasa*, *Mvh* was used to knock in the reporters, *LacZ* and *GFP*. In this study, germ cells were generated in embryoid bodies, which were detected through the expression of MVH-GFP. This process was greatly enhanced by the exposure of ES cells to BMP4. These MVH-GFP cells were aggregated with E12.5–E13.5 male gonadal cells when germ cells within these aggregates developed into elongated spermatids.

These studies show that it may be possible to generate an efficient *in vitro* system to derive germ cells from pluripotent ES cells. Such a system would be useful to study the mechanism of PGC specification *in vitro*, as well as other aspects, including the formation of gametes, and aspects of epigenetic reprogramming of the genome. Derivation of germ cells from human ES cells would be particularly useful for studies on this lineage. Furthermore, derivation of human oocytes from ES cells would greatly add to this scarce resource, thus providing opportunities for fundamental studies on somatic cell reprogramming, and for the subsequent derivation of stem cell lines from human somatic cells for investigations of specific mutations and diseases.

#### *31.2.1.2 From Germ Cells to Stem Cells*

Embryonal carcinoma cells (EC) that are derived from PGCs *in vivo* were the first pluripotent stem cells to be identified, and several loci have been identified that have a critical role in this process. At the same time, derivation of pluripotent cells from PGCs has also been achieved *in vitro*. This conversion of germ cells into EG cells occurs in the presence of leukemia inhibitory factor (LIF), basic fibroblast growth factor (FGF2), and the Kit ligand (KL). The precise mechanism for conversion of the highly specialized germ cells into pluripotent stem cells is largely unknown. Further investigations would provide insights into dedifferentiation of cells, and on the mechanism of genomic reprogramming.

### 31.2.2 Epigenetic Reprogramming in Germ Cells

One of the properties of germ cells, of particular interest, is the epigenetic reprogramming of the genome. This event occurs when PGCs enter into the

developing gonads (Figure 31.2), when there is extensive erasure of epigenetic modifications, including erasure of genomic imprints and reactivation of the inactive X-chromosome. New parental imprints are initiated later during gametogenesis, particularly oogenesis, and these modifications that are heritable after fertilization dictate parent-of-origin-dependent gene expression.

As germ cells proliferate after the formation of the founder population at E7.5, they start to migrate to the developing gonads. At this stage, germ cells, as well as somatic cells, contain epigenetic marks associated with imprinted genes. During their migration, female germ cells also show inactivation of one X-chromosome. Upon the entry of germ cells into developing gonads at E10.5–E11.5, a major epigenetic reprogramming event occurs, which includes reactivation of the inactive X-chromosome, and the erasure of epigenetic marks associated with imprinted genes. Indeed, there appears to be genome-wide DNA demethylation of the genome at this time. This genomic reprogramming event occurs relatively rapidly, and it is completed by E12.5.

The mechanism involved in the erasure of epigenetic modifications in the germ line may provide insights into the erasure of epigenetic modifications from somatic nuclei that occur after transplantation into oocytes, which restores totipotency. If this is the case, similar factors may be transcribed during oocyte maturation at the germinal vesicle stage and translated, to be stored in oocytes as maternally inherited factors. The onset of genomic reprogramming in PGCs in the gonad may be triggered by a signal from somatic cells, or in response to a developmental timer, such as the number of cell divisions in PGCs since the establishment of the founder population of PGCs. Because the gonads at E11.5 are bipotent, it is possible that the signal from somatic cells, if it exists, should be the same in both male and female embryos. In this case, it would be of interest to discover the nature of the signal and determine how such an external cue can trigger extensive genomic reprogramming. However, there is also support for the alternative developmental timer model, since EG cells show erasure of imprints even when they are derived from PGCs where this process has not yet commenced. It is possible that erasure is initiated when germ cells complete a critical number of cell cycles, although, as these cells are cultured in a complex medium, a role for an environmental cue cannot be entirely discounted. Whatever the case may be, it is important to note that EG cells themselves have the property to induce erasure of epigenetic modifications from somatic nuclei (see below).

#### *31.2.2.1 Genomic Reprogramming in Oocytes*

Resumption of oocyte growth is accompanied by further epigenetic reprogramming events, particularly the initiation of genomic imprints. The majority of the epigenetic marks associated with imprinting are introduced during oocyte growth, although some genes acquire paternal-specific imprints in

the male germ line. These epigenetic marks are eventually detected as DNA methylation of specific *cis*-control elements. Some of the marks, for example, in the *Igf2r* locus, ensure that the gene will be active only when maternally inherited, while others such as *Peg3* will be silent in the female genome. *Dnmt3l* is a key gene involved in the initiation of parental imprints, which acts together with the *de novo* DNA methylase enzyme, Dnmt3a. A mutation in the *Dnmt3l* gene does not disrupt development or maturation of the oocyte, except that these oocytes do not carry appropriate maternal imprints or epigenetic marks. Following their fertilization, the resulting embryos are unable to develop normally, and they die shortly after implantation. Other genes, such as *H19*, undergo DNA methylation in the paternal germ line, and this gene is repressed in the paternal genome. This topic is discussed comprehensively elsewhere.

### 31.2.3 Maternal Inheritance and Reprogramming of Parental Genomes

As in other organisms, mouse oocytes contain a number of maternally inherited proteins and messages (Figure 31.2). In mammals, maternally inherited factors are essential for totipotency and pluripotency, such as Oct4, Esg1, and Stella, although there is no Nanog. Maternal inheritance of Stella is apparently necessary for normal preimplantation development. The oocytes also contain epigenetic modifiers, including the *Polycomb* group proteins, Ezh2 and eed, as well as the heterochromatin factor, HP1. These factors are essential for regulating early development, and for generating the pluripotent epiblast and trophectoderm cells of blastocysts. The oocyte is also likely to inherit some key chromatin remodeling factors.

In mammals, the parental genomes exhibit epigenetic asymmetry in the zygote as a result of imprinting, which confers functional differences between parental genomes. At fertilization, the maternal genome apparently has high methylated lysine 9 histone H3 (H3meK9). Immediately after fertilization, the hetrochromatin protein HP1b binds preferentially to the maternal genome. The Polycomb proteins, Ezh2 and eed, also bind preferentially to the maternal genome. While this takes place, the paternal genome that has relatively low levels of H3meK9 shows genome-wide DNA demethylation, thus enhancing the epigenetic differences between the parental genomes. Ezh2 has the conserved suvar E2 trithorax (SET) domain with histone methylase activity for methylation of histone H3-lysine 27/lysine 9 (H3meK27/9). The maternal inheritance of Ezh2 *per se* is apparently important, since depletion of this factor from oocytes results in development of very small neonates, presumably because of an effect on placental development. This seems likely because the neonates eventually grow and acquire normal size, indicating a placental functional deficiency during development. Whether this is due to an

effect on imprinted genes remains to be determined. These experiments show that factors present within the oocyte have the potential to exert a variety of epigenetic effects on development. Somatic nuclei transplanted into oocytes would be affected by the activities of these factors during reprogramming, but the variable expression of genes associated with totipotency and of imprinted genes argues that appropriate epigenetic reprogramming of the genome may not be accomplished in every case (see below).

Factors involved in chromatin remodeling are also likely to be important for early development and genomic reprogramming, as they regulate accessibility to DNA. The SWI/SNF-like complexes consist of at least two ATPase subunits, BRG1 and BRM. *Brg1* is important during preimplantation development, as loss of function is lethal during this phase. It is also known that mutation in *ATRX*, a member of the SNF2 helicase/ATPase family, has an effect on DNA methylation of highly repeated sequences. Mutation in *Lsh* similarly results in substantial demethylation of the genome. Lsh is related to the SNF2 subfamily; most members of the SNF2 family of proteins appear to have the capacity to alter chromatin structure. The activity of the nucleosome-dependent ATPase, ISWI, may be used in chromatin remodeling in nuclear reprogramming of somatic nuclei; if so, it is likely to be present in the oocyte, and would have a role in the zygote. One of the earliest changes observed following fertilization (or indeed after transplantation of the somatic nucleus) is the apparent increase in the size of the nucleus. This morphological change may be in response to chromatin remodeling factors belonging to the ISWI complexes. This activity may be necessary for the initial unwinding of the chromosomes, to facilitate epigenetic modifications of the chromatin.

### 31.2.4 Reprogramming During Early Development

Epigenetic reprogramming of the embryonic genome continues throughout preimplantation development, as judged by the continual changes in histone modifications and a decline in the genomic levels of DNA methylation. During preimplantation development, both pluripotent epiblast and differentiated trophectoderm cells are formed. There are differences in the epigenetic reprogramming in these two tissues. For example, there is preferential paternal X-inactivation in the trophectoderm, a process in which the *Polycomb* group proteins, Ezh2/eed complex, have a significant role. The cells of the late morula, which are positive for the expression of *Nanog*, and destined to form the inner cell mass, cease to show Ezh2/eed accumulation at Xi, as seen with the paternal X-chromosome in the trophectoderm. Ezh2 is also detected in the inner cell mass, which may account for the presence of the overall H3meK27 staining of epiblast cells, which are positive for Oct4 expression.

It appears that histone modifications, such as H3meK27, may have a role in the maintenance of epigenetic plasticity of the pluripotent epiblast cells, since

the loss of function of Ezh2 is early-embryonic-lethal, and it is not possible to derive pluripotent ES cells from blastocyst that are null for Ezh2. These experiments show the importance of appropriate epigenetic reprogramming of the genome for early development, and for generating pluripotent epiblast cells that are the precursors of both somatic and germ cell lineages. As we learn more about nuclear reprogramming events which occur normally in germ cells, oocytes, and early development, these studies are likely to be used to identify key candidates for genomic reprogramming.

## 31.3 REPROGRAMMING SOMATIC NUCLEI

### 31.3.1 Nuclear Transplantation

Epigenetic reprogramming of somatic nuclei transplanted into oocytes must require the erasure and initiation of appropriate epigenetic modifications compatible for development. This subject has already been reviewed extensively elsewhere. At least some of the key reprogramming events may be faulty, to account for the very low success rate, since somatic nuclei undergo variable reprogramming resulting in a wide variety of phenotypes. The effects of aberrant reprogramming are apparent, particularly soon after implantation and during postimplantation development. Both the embryo and extraembryonic tissue seem to be affected. Some epigenetic marks associated with imprinted genes are erased, resulting in the aberrant expression of these genes. It seems likely, therefore, that a large number of genes fail to show appropriate temporal and spatial patterns of expression. Further studies on the mechanisms of genomic reprogramming during normal development and following nuclear transplantation are necessary to assess the reasons for faulty reprogramming of somatic nuclei.

### 31.3.2 Reprogramming in Embryonic Stem–Somatic and Embryonic Germ–Somatic Cell Hybrids

Somatic nuclear reprogramming has also been demonstrated in hybrid cells between pluripotent ES/EG and somatic cells, which also restores pluripotency in somatic nuclei. These studies indicate that not only the oocytes, but also pluripotent ES/EG cells, must contain appropriate factors to reprogram the somatic nucleus. Reprogramming of somatic nuclei in ES/G–somatic cell hybrids is, however, relatively less complex compared to its transplantation into oocytes. This is because the somatic nucleus in the oocyte has to be reprogrammed to recapitulate the entire program of early development to the blastocyst stage. It is important to note that this donor somatic nucleus has to be reprogrammed to generate pluripotent epiblast cells, as well as the highly differentiated trophectoderm cells. The latter should be viewed as a transdifferentiation event, because somatic nuclei of diverse origin must direct

differentiation of highly specialized trophectoderm cells after only a few cleavage divisions. Indeed, in some respects, this transdifferentiation event is more striking as a reprogramming event. By comparison, reprogramming of somatic nuclei in ES/EG–somatic cell hybrids is less complex, as there is restoration of pluripotency without the necessity to recapitulate early events of development.

Although EG and ES cells on the whole have similar effects on somatic nuclei, there is at least one critical difference between them. Using EG–thymocyte hybrid cells, it was shown that the somatic nucleus underwent extensive reprogramming, resulting in the erasure of DNA methylation associated with imprinted genes, and the inactive X-chromosome was reactivated. The somatic nucleus also acquired pluripotency, as judged by the activation of the *Oct4* gene, and the hybrid cells could differentiate into all three germ layers in chimeras. This study shows that EG cells, apart from conferring pluripotency to the somatic nucleus, retained a key property found only in germ cells, which is the ability to erase parental imprints and, indeed, induce genome-wide DNA demethylation. Experiments using ES–thymocyte hybrid cells gave similar results, including the restoration of pluripotency to somatic nuclei, as shown by the activation of the Oct4-GFP reporter gene, and for the ability of these cells to differentiate into a variety of cell types. Unlike EG cells, however, ES cells do not cause erasure of imprints from somatic nuclei. Furthermore, in ES–EG hybrids, EG cells can induce erasure of imprints from ES cells, which shows that EG cells have dominant activity for the erasure of imprints and DNA demethylation. However, from these studies it is clear that DNA demethylation activity, at least for the erasure of imprints present in EG cells, is not essential for restoring pluripotency to somatic cells. It is possible to use this system to design cell-based assays in search of key reprogramming factors.

The ability of ES/EG cells to restore pluripotency in somatic nuclei is significant because it also opens up possibilities to identify the molecules involved in reprogramming somatic nuclei. Such studies are difficult with mammalian oocytes, partly because they are small compared to amphibian oocytes, and it is difficult to collect large numbers of them. More importantly, as discussed earlier, oocytes are complex cells containing factors essential for pluripotency, as well as for the early development and differentiation of trophectoderm cells. By contrast, pluripotent ES/EG cells are relatively less complex, and, more importantly, they can be grown indefinitely *in vitro*. Thus, they can provide a considerable source of material for analysis. For example, it is possible to use nuclear extracts from ES/EG cells to examine reprogramming of somatic nuclei, as described in one experimental approach. The availability of relatively large amounts of nuclear extracts from ES/EG cells also makes it possible to undertake biochemical studies to identify the key reprogramming factors.

## 31.4 CONCLUSIONS

The evidence from studies on early mammalian development shows that there is dynamic and extensive reprogramming of the genome in the oocyte, zygote, and germ cells. Pluripotent stem cells also appear to show considerable potential for genomic reprogramming, and while there are differences between ES and EG cells, both of them can restore pluripotency to somatic nuclei. There is maternal inheritance of factors for pluripotency, epigenetic modifications, and chromatin remodeling in the oocyte. Reprogramming in the oocyte is relatively complex, since the parental pronuclei exhibit epigenetic asymmetry in the zygote. The paternal genome becomes rapidly demethylated after fertilization, but the maternal genome does not, at least in part, because of the differential histone modifications, such as the preferential H3meK9 of the maternal genome, but not of the paternal genome, and the preferential binding of HP1b and Ezh2/eed proteins to it. It is possible that epigenetic reprogramming of somatic nuclei may be affected by their original epigenetic state.

The phenomenon of reprogramming somatic nuclei is now well established in mammals, both by nuclear transplantation studies and in heterokaryons, but the mechanisms and the key molecules involved are yet unknown. It is reasonable to assume that some of the factors involved in reprogramming the genome in the germ line are also present in the oocyte. It is also possible that some of the basic reprogramming factors present in pluripotent stem cells are also present in the oocyte.

A likely sequence of events for converting a somatic nucleus to a pluripotent nucleus may first require chromatin remodeling activity. Many of these complexes are known to exist in mammals, but at this stage it is not known precisely which are important for reprogramming of somatic nuclei. This may be followed by changes in histone modifications compatible with pluripotency. What these changes are has yet to be fully determined, together with the identity of the histone modifiers.

As ES/EG cells apparently have the capacity for reprogramming somatic nuclei to pluripotency, they may be used for identifying key molecules necessary for genomic reprogramming through cell-based assays, combined with appropriate biochemical and cellular analyses.

## FOR FURTHER STUDY

[1] Collas P. Nuclear reprogramming in cell-free extracts. Philos Trans R Soc Lond B Biol Sci 2003;358(1436):1389–95.

[2] Donovan PJ, de Miguel MP. Turning germ cells into stem cells. Curr Opin Genet Dev 2003;13(5):463–71.

[3] Li E. Chromatin modification and epigenetic reprogramming in mammalian development. Nat Rev Genet 2002;3(9):662–73.

[4] McLaren A. Primordial germ cells in the mouse. Dev Biol 2003;262(1):1–15.

[5] Reik W, Walter J. Genomic imprinting: parental influence on the genome. Nat Rev Genet 2001;2(1):21–32.

[6] Saitou M, Payer B, Lange UC, Erhardt S, Barton SC, Surani MA. Specification of germ cell fate in mice. Philos Trans R Soc Lond B Biol Sci 2003;358(1436):1363–70.

[7] Surani MA. Reprogramming of genome function through epigenetic inheritance. Nature 2001;414(6859):122–8.

[8] Surani MA. Stem cells: how to make eggs and sperm. Nature 2004;427(6970):106–7.

[9] Tada T, Tada M. Toti-/pluripotential stem cells and epigenetic modifications. Cell Struct Funct 2001;26(3):149–60.