CHROMATIN MODIFICATION AND EPIGENETIC REPROGRAMMING IN MAMMALIAN DEVELOPMENT

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The developmental programme of embryogenesis is controlled by both genetic and epigenetic mechanisms. An emerging theme from recent studies is that the regulation of higher-order chromatin structures by DNA methylation and histone modification is crucial for genome reprogramming during early embryogenesis and gametogenesis, and for tissue-specific gene expression and global gene silencing. Disruptions to chromatin modification can lead to the dysregulation of developmental processes, such as X-chromosome inactivation and genomic imprinting, and to various diseases. Understanding the process of epigenetic reprogramming in development is important for studies of cloning and the clinical application of stem-cell therapy.

HISTONES
Small, highly conserved basic proteins, found in the chromatin of all eukaryotic cells, which associate with DNA to form a nucleosome.

CORE HISTONES
These are histones H2A, H2B, H3 and H4. A nucleosome contains two copies of each of the core histones wrapped by 146-bp DNA.

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It is well known that DNA and HISTONES are the basic components of a chromosome, in which the DNA helix is wrapped around CORE HISTONES to form the simple 'beads on a string' structure that is then folded into higher-order chromatin. Chromatin also contains various proteins that are required for its assembly and packaging, and for DNA replication, DNA and histone modification and transcription, and DNA repair and recombination. Chromatin is not uniform with respect to gene distribution and transcriptional activity. It is organized into domains, such as EUCHROMATIN and HETEROCHROMATIN, which have different chromosomal architecture, transcriptional activity and replication timing.

How chromatin maintains its architecture and biological properties over many cell generations and how transcription factors find their target sequences in the nucleus are not fully understood. EPIGENETIC marking by the covalent modification of DNA and of the core histones creates molecular landmarks that differentiate between active and inactive chromatin. Histone modification seems to be a universal regulatory mechanism among eukaryotic organisms from yeast to human. DNA methylation, however, is less conserved, but is a common and rapidly evolving mechanism among higher eukaryotic organisms with more complex genomes.

The principal epigenetic mechanisms by which tissue-specific gene-expression patterns and global gene silencing are established and maintained are CHROMATIN MODIFICATION and CHROMATIN REMODELLING. Several recent reviews have covered in detail the mechanisms and functions of various epigenetic processes in gene regulation[1–4]. This review focuses on recent insights into the roles of DNA methylation and histone modification in mammalian development. Of particular interest are recent reports of the epigenetic regulation of genomic imprinting, X inactivation and genome reprogramming in normal, knockout and cloned embryos, and the integration of epigenetic mechanisms into these processes.

The interplay of epigenetic regulators

The covalent modification of NUCLEOSOMAL DNA and core histones, and ATP-dependent chromatin remodelling, are important in the regulation of gene expression, DNA replication and many other biological processes. The proteins that carry out these modifications and chromatin remodelling are listed in BOX 1. Increasing evidence indicates that these general chromatin-modification and chromatin-remodelling proteins do not act alone, but interact with one another, often by forming large protein complexes that regulate

higher-order chromatin structures and the accessibility of chromatin to various factors (FIG. 1). The stable inheritance of chromatin structure, and changes to its accessibility, are likely to be essential for all chromatin-associated biological processes.

DNA methylation and histone modification serve as epigenetic marks for active or inactive chromatin, and such epigenetic marks are heritable. In mammalian cells, DNA methylation occurs predominantly at CpG dinucleotides and is catalysed by two important classes of DNA methyltransferases (BOX 1). DNA methyltransferase 1 (Dnmt1) is a maintenance enzyme that methylates hemi-methylated CpG dinucleotides in the nascent strand of DNA after DNA replication[5], and its function is essential for maintaining DNA-methylation patterns in proliferating cells[6]. Dnmt3a and Dnmt3b are required for the initiation of *de novo* methylation *in vivo* and for establishing new DNA-methylation patterns during development[7–9]. Both Dnmt1 and Dnmt3a have been shown to interact with histone deacetylases (HDACs) and can repress transcription[10].

DNA methylation regulates gene expression through several distinct mechanisms. DNA methylation can directly block transcription regulatory factors from binding to their target sequences, although regulation by such a mechanism *in vivo* is relatively rare. A recent study has shown that the gene that encodes the glial fibrillary acidic protein (GFAP) is activated during astrocyte differentiation by the demethylation of a CpG dinucleotide that lies in its promoter region in the STAT3 (signal transducer and activator of transcription 3)-binding element[11]. DNA methylation can also repress gene expression through several methyl-CpG-binding proteins (MECPs) that 'read' DNA-methylation patterns (BOX 1). For instance, MECP2 forms a complex with HDACs and a co-repressor protein, Sin3a, to repress transcription in a methylation-dependent manner[12,13]. Another methyl-CpG-binding protein MBD2 forms a complex with the multisubunit NuRD complex, which contains an ATP-dependent chromatin-remodelling protein, Mi-2, and HDACs[14,15]. The MBD2–NuRD complex (previously known as MeCP1) can repress methylated promoters and remodel methylated chromatin with high efficiency[16,17]. The MECP2–Sin3a–HDAC and MBD2–NuRD complexes provide a mechanistic link between DNA hypermethylation and histone deacetylation in transcriptional repression. However, it is noteworthy that DNA methylation is not

EUCHROMATIN
The lightly staining regions of the nucleus that generally contain decondensed, transcriptionally active regions of the genome.

HETEROCHROMATIN
A cytologically defined genomic component that contains repetitive DNA (highly repetitive satellite DNA, transposable elements and ribosomal DNA gene clusters) and some protein-coding genes.

EPIGENETIC
Any heritable influence (in the progeny of cells or of individuals) on chromosome or gene function that is not accompanied by a change in DNA sequence. Examples of epigenetic events include mammalian X-chromosome inactivation, imprinting, centromere inactivation and position effect variegation.

CHROMATIN MODIFICATION
Includes processes such as DNA methylation and histone modification (acetylation, phosphorylation, methylation and ubiquitylation).

CHROMATIN REMODELLING
Transient changes in chromatin accessibility.

NUCLEOSOME
The fundamental unit into which DNA and histones are packaged in eukaryotic cells. It is the basic structural subunit of chromatin and consists of 200 bp of DNA and an octamer of histone proteins, comprising two of each core histone.

Box 1 | Components of the main chromatin-modification and -remodelling complexes

DNA cytosine methyltransferases

Three active DNA cytosine methyltransferases — Dnmt1, Dnmt3a and Dnmt3b — have been identified in mammals. They contain characteristic and highly conserved cytosine-methyltransferase motifs in the catalytic domain. Dnmt1 is ubiquitously expressed and is a maintenance methyltransferase, which restores DNA-methylation patterns by methylating hemi-methylated CpG sites (FIG. 1) after DNA replication[5]. Dnmt3a and Dnmt3b are regulated during development and are required to initiate *de novo* methylation and establish new DNA-methylation patterns[7]. The activity and function of a putative cytosine methyltransferase — Dnmt2 — remain undefined[115].

Methyl-CpG-binding proteins

Six methyl-CpG-binding proteins, including MECP2, MBD1, MBD2, MBD3, MBD4 and Kaiso, have been identified in mammals. MECP2 binds methylated DNA *in vitro* and *in vivo*. It contains a methyl-CpG-binding domain (MBD) at its amino terminus and a transcription repression domain (TRD) in its middle. MBD1–4 were cloned on the basis of their sequence homology to MECP2 in the MBD, and all except MBD3 bind methyl CpG preferentially[1]. *Xenopus* MBD3 can bind methyl CpG[15]. MBD3 is an integral component of the Mi-2–NuRD complex (see below)[14,15]. MECP2, MBD1 and MBD2 function as transcription repressors. MBD4 is a DNA glycosylase and is involved in DNA mismatch repair[116,117]. Kaiso, which lacks an MBD domain, binds methylated CGCG through its zinc-finger domain[118]. Different methyl-CpG-binding proteins might recruit different chromatin-remodelling proteins and transcription-regulatory complexes to methylated DNA targets in the genome.

Histone-modification enzymes

The modification of core histones at their amino-terminal tails by acetylation, phosphorylation, methylation and ubiquitylation has a fundamental role in gene regulation. The combined acetylation, phosphorylation and methylation status of the histone tails determines gene activity[2,3,20]. Several classes of histone methyltransferases have been identified[3], most notably an H3-K4 methyltransferase[119,120] and five H3-K9 methyltransferases, Suv39h1 and Suv39h2 (REF. 121), G9a[37], ESET/SetDB1 (REFS 122,123) and Eu-HMTase1 (REF. 124). Several transcription co-activators, such as Gcn5, p300/CBP, PCAF, TAF250 and the p160 family nuclear receptor, that have intrinsic histone acetyltransferase (HAT) activity, and several histone deacetylases (HDACs), have also been identified in mammalian cells.

ATP-dependent remodelling complexes

Three classes of chromatin-remodelling protein complexes — SWI/SNF/Brm, ISWI and Mi-2/NuRD — which are present in mammalian cells, contain different catalytic ATPase subunits and associated proteins[4]. Chromatin-remodelling enzymes use the energy of ATP hydrolysis to introduce superhelical torsion into nucleosomal DNA, which leads to the formation of nucleosomes that contain exposed DNA bulges or loops[125]. Such transient conformational changes in nucleosomal DNA can alter the accessibility of chromatin to various chromatin proteins that control transcription, DNA replication, recombination and other biological processes.

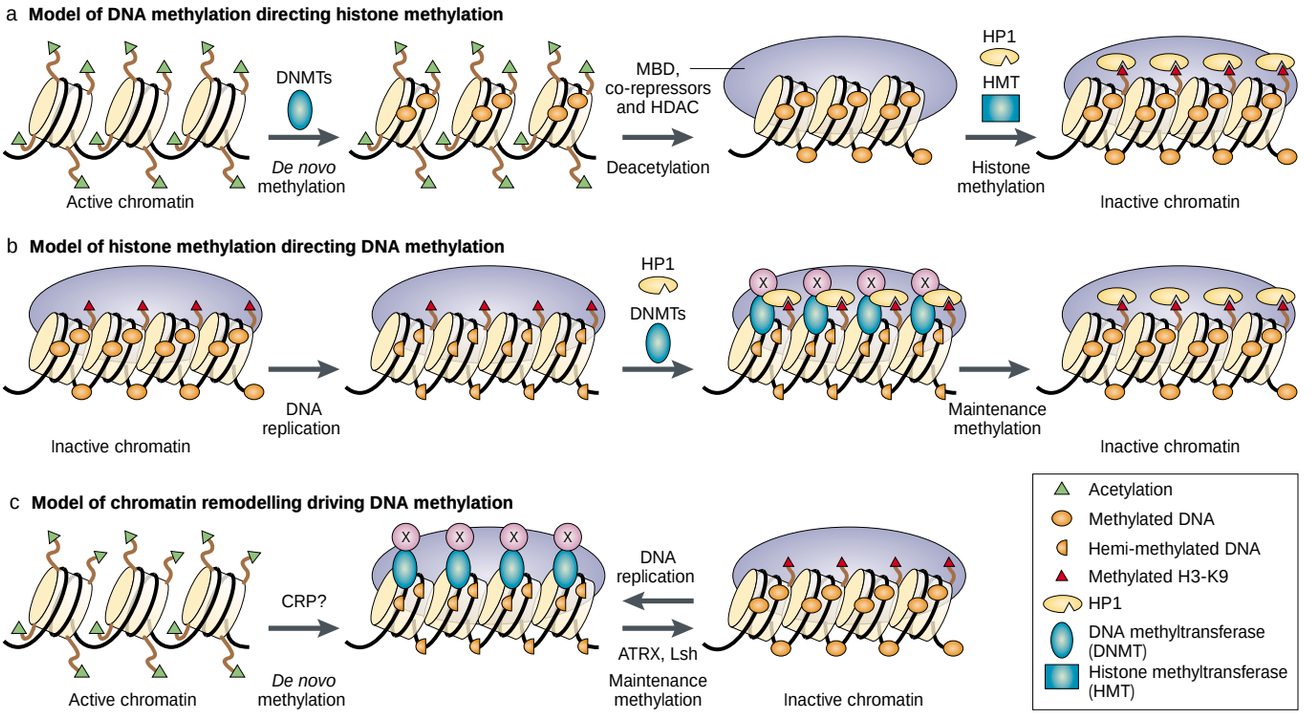

Figure 1 | **Links between DNA methylation, histone modification and chromatin remodelling.** In mammalian cells, both DNA methylation and histone modification are involved in chromatin silencing. DNA methylation and histone modification are believed to be interdependent processes. Three possible models of how they might influence each other are shown. **a** | A model of DNA methylation directing histone methylation. DNA methylation patterns are established through *de novo* methylation by the DNA methyltransferases DNMT3A and DNMT3B, and are maintained by DNMT1. Methyl-CpG-binding proteins (MBD) and histone deacetylase (HDAC) complexes, such as the MECP2–Sin3a–HDAC complex, are believed to then be recruited to the methylated region to induce histone deacetylation and silencing[12,13]. The chromatin then attracts histone methyltransferases (HMTs), such as Suv39h or G9a, which methylate the lysine 9 residue on histone H3 (H3-K9) and stabilize the inactive state of the chromatin[36,37]. **b** | A model of histone methylation directing DNA methylation. Methyl H3-K9 acts as a signal for inactive chromatin by recruiting HP1 to methylated histones, which might in turn recruit DNA methyltransferases directly or indirectly (through an unknown factor, factor X) to the silent chromatin to maintain DNA methylation and stabilize the inactive chromatin[21,22]. **c** | A model of chromatin remodelling driving DNA methylation. The ATP-dependent chromatin-remodelling and DNA-helicase activities of proteins, such as ATRX and Lsh, might facilitate DNA methylation and histone modification by unwinding nucleosomal DNA to increase its accessibility to DNMTs, HDACs and HMTs. The disrupted function of these proteins impairs both DNA methylation and histone methylation, as has been shown in plants[23,24]. The chromatin-remodelling protein (CRP) that is involved in *de novo* methylation has yet to be identified.

always associated with gene silencing. One interesting study has shown that DNA methylation can augment expression of insulin-like growth factor 2 (*Igf2*) by blocking the binding of repressor proteins to a silencer element in the gene[18,19].

The modification of core histones at the lysine, arginine and serine residues that lie in their amino-terminal tails is far more complex, involving many histone-modification enzymes (BOX 1). For example, Lys9, 14, 18 and 23 of H3 and Lys5, 8, 12 and 16 of H4, together with lysines on H2A and H2B, can be acetylated; whereas Lys4, 9 and 27, Arg2, 17 and 26 of H3, and Lys20 and Arg3 of H4 can be methylated. Histone acetylation and deacetylation have been shown to determine the transcriptional activity of the chromatin[20]. Recent studies of the methylation of Lys4 and 9 of H3 have provided exciting new insights into the role of histone methylation in epigenetic regulation and into the interactions between DNA methylation and histone methylation.

Methylation of Lys4 of H3 (H3-K4) has been associated with active gene expression, whereas methylation of Lys9 of H3 (H3-K9) has been associated with transcriptional silencing[2,3]. The link between DNA methylation and histone methylation first came from a study in a filamentous fungus, *Neurospora crassa*, which showed that mutations of the *dim-5* (*defective in methylation 5*) gene, which encodes a SET-DOMAIN-containing H3-K9 methyltransferase, resulted in the complete loss of all forms of DNA methylation[21]. Similar observations were made in *Arabidopsis thaliana*, in which mutations of an H3-K9 methyltransferase (encoded by *kryptonite*) abolished methylation of CpNpG sites, but not CpG sites[22]. Biochemical analysis further showed that histone methylation might regulate DNA methylation in *Arabidopsis* through HETEROCHROMATIN PROTEIN 1 (HP1), which binds to methylated H3-K9 through its CHROMODOMAIN and recruits the DNA methyltransferase Cmt3 to its target CpNpG sites[22]. However, how histone

SET DOMAIN
(Suvar3–9, Enhancer-of-zeste, Trithorax) domain. An evolutionarily conserved sequence motif that was initially identified in the *Drosophila* position effect variegation suppressor Su(var)3–9, the Polycomb-group protein Enhancer-of-zeste, and the Trithorax-group protein Trithorax. It is present in many histone methyltransferases and is required for enzyme activity.

HETEROCHROMATIN PROTEIN 1 (HP1). A protein that binds to highly repetitive, heterochromatic satellite DNA at centromeres and telomeres.

methylation regulates CpG methylation is not known. It also remains to be determined whether the maintenance of DNA-methylation patterns depends on histone methylation and vice versa in mammalian cells. Because plant and mammalian cells contain several CpG methyltransferases and H3-K9 methyltransferases, the interaction is likely to be more complicated than that in *Neurospora*. The molecules that target DNA methyltransferases and histone methyltransferases to specific genomic loci also remain to be discovered.

Three classes of ATP-dependent chromatin-remodelling proteins are present in mammalian cells (BOX 1), which use the energy of ATP hydrolysis to introduce transient changes in nucleosomal DNA that make chromatin accessible to other proteins, such as transcription factors[4]. In addition to modulating accessibility to transcription factors, chromatin remodelling is also required for DNA methylation and histone modification. In *Arabidopsis*, the *DECREASE IN DNA METHYLATION 1* (*DDM1*) gene, which encodes an SNF2-like protein (BOX 1), is required for maintaining DNA methylation[23]. Mutations in *DDM1* also result in a reduction of H3-K9 methylation and in an increase of H3-K4 methylation in the heterochromatin[24]. The studies of two SNF2 family proteins, ATRX (α-thalassaemia/mental retardation syndrome, X-linked) and Lsh (lymphoid-specific helicase; the mammalian homologue of DDM1), have revealed that proteins with chromatin-remodelling and DNA-helicase activities can also modulate DNA methylation in mammalian cells[25,26]. How chromatin-remodelling proteins interact with DNA methyltransferases and histone methyltransferases to bring about DNA methylation and histone methylation, respectively, will be an active area of future research.

Gene-targeting studies in mice have shown that chromatin-modification enzymes and chromatin-remodelling proteins have a pivotal role in mammalian

Table 1 | Knockout mice with defective chromatin modification

Gene/protein	Mutant phenotype	References
DNA methylation		
Dnmt1$^{-/-}$	Genome-wide demethylation and developmental arrest at E8.5	6,64
Dnmt3a$^{-/-}$	Malfunction of the gut, spermatogenesis defect, death at ~4 weeks of age	7
Dnmt3b$^{-/-}$	Demethylation of minor satellite DNA, mild neural tube defects and embryonic lethality at ~E14.5–E18.5	7
Dnmt3a$^{-/-}$,*3b*$^{-/-}$	Failure to initiate *de novo* methylation after implantation and developmental arrest at E8.5	7
Dnmt3l$^{-/-}$	Failure to establish maternal methylation imprints in oocytes and male sterility due to spermatogenesis defects	52,53
Mbd proteins		
Mbd2$^{-/-}$	Viable and fertile, but showing defective maternal behaviour	28
Mbd3$^{-/-}$	Normal implantation, developmental arrest at E6.5 or earlier	28
Mecp2$^{-/-}$	Complex neurological defects, including tremors, ataxia, hind-limb clasping, stereotypic forelimb motions, increased anxiety-related behaviour and seizures	33–35
Histone modification enzymes		
G9a$^{-/-}$	Loss of H3-K9 methylation in euchromatin, developmental and growth arrest at E8.5	37
Hdac1$^{-/-}$	Severely reduced growth and embryonic lethality at ~E9.5	32
Suv39h1$^{-/-}$,2$^{-/-}$	Loss of H3-K9 methylation in heterochromatin, polyploidy in MEF cells, chromosome-pairing defects during spermatogenesis, male sterility and death of some double-mutant embryos at ~E14.5	36
SWI2/SNF2 complex proteins		
Lsh$^{-/-}$	Global demethylation of genomic DNA at E13.5 and postnatal lethality	26
Brm$^{-/-}$	Viable and normal, slightly overweight	126
Brg1$^{-/-}$	Growth defects of primitive ectoderm and trophectoderm, peri-implantation lethality	27
Snf5$^{-/-}$	Peri-implantation lethality	127
Srg3$^{-/-}$	Defective inner cell mass outgrowth, death shortly after implantation	128
Polycomb group proteins		
Eed$^{-/-}$	Defective gastrulation and failure to maintain the inactive X in trophoblast cells	31,94
Ezh2$^{-/-}$	Growth defect of the primitive ectoderm and peri-implantation lethality	29
Yy1$^{-/-}$	Defects in epiblast cell growth or survival, and peri-implantation lethality	30

E, embryonic day. Brg1, brahma-related gene 1; Brm, brahma; *Dnmt*, DNA methyltransferase; *Dnmt3l*, Dnmt3-like; Eed, embryonic ectoderm development; Ezh2, enhancer of zeste homologue 2; Hdac1, histone deacetylase 1; Lsh, lymphoid-specific helicase; *Mecp2*, methyl-CpG-binding protein 2; MEF, mouse embryonic fibroblast; Snf5, integrase interactor 1; Srg3, SWI3-related gene product; Suv39h, homologue of Su(var)3-9; Yy1, Yin Yang 1.

CHROMODOMAIN
A highly conserved sequence motif that has been identified in various animal and plant species. Chromodomain proteins are often structural components of large macromolecular chromatin complexes or are involved in remodelling chromatin structure.

development. The functions of some of these proteins in mouse development, as defined by gene-knockout experiments, are summarized in TABLE 1. Some genes, such as *Brg1* (REF. 27), *Mbd3* (REF. 28), *Ezh2* (REF. 29) and *Yy1* (REF. 30), are required for peri-implantation or early post-implantation development, whereas others, such as *Eed*[31], *Dnmt1* (REF. 6), *Dnmt3a*/*3b* (REF. 7) and *Hdac1* (REF. 32), are required for embryonic growth during or after GASTRULATION. Surprisingly, the genes that encode Mecp2 and Mbd2 are not essential for mouse embryonic development. However, both are required for proper brain functions after birth, and mutations of *MECP2* in humans cause a neurological and behavioural disorder known as Rett syndrome[28,33–35]. The overlapping functions of methyl-CpG-binding proteins, such as MBD1, MBD2 and MECP2, might explain the lack of early developmental phenotypes. Targeted inactivation of the histone methyltransferase genes *Suv39h1* and *Suv39h2* in mice results in the death of some, but not all, double-mutant embryos[36], whereas the inactivation of *G9a*, which encodes another histone H3-K9 methyltransferase, leads to early embryonic lethality[37]. HP1 has been shown to bind with high affinity to methylated H3-K9, and such interactions are crucial for heterochromatin formation through the oligomerization of HP1 proteins[38–40]. However, the function of HP1 family proteins in mammalian development has yet to be determined.

The interplay of various chromatin-modification and -remodelling proteins in establishing and maintaining chromatin structures and activities will continue to be the focus of future studies. Many important components of this process, including particularly RNA and protein factors that target chromatin-modification enzymes to specific genomic loci, remain to be discovered. Well-defined developmental processes, such as genomic imprinting, X inactivation and tissue differentiation, provide an excellent opportunity for studying the epigenetic regulation of gene expression during mammalian development.

Epigenetic reprogramming in gametes

Gametes are terminally differentiated and highly specialized cells that carry all the information that is necessary for the initiation of a new life cycle after normal fertilization. Nuclear-transplantation experiments have shown that both the maternal and paternal genomes are necessary for embryonic development as they are programmed differently and are functionally non-equivalent[41,42]. The functional differences between the paternal and maternal genomes are attributed to the differential expression of the paternal and maternal alleles of several dozen imprinted genes during development. These differences originate from the differential modification of the genome (or genomic imprinting) in the male and female gametes[43]. The paternal alleles of the *H19* and *Rasgrf1* genes are methylated in their 5′ upstream regions in the male germ cells during embryogenesis, whereas the other known imprinted genes, such as *Igfr2* and *Snprn*, acquire their methylation imprints from the oocyte; the deletion of such DIFFERENTIALLY METHYLATED REGIONS results in the loss of imprinting[44–49]. The gamete-derived methylation patterns of imprinted genes are maintained in the somatic tissues throughout embryonic development, but are erased in the primordial germ cells[50,51]; therefore, genomic imprinting can be reversed in the germ line.

Imprinting in oocytes. The genetic evidence that DNA methylation is an essential epigenetic mark for the establishment of genomic imprinting comes from recent studies of the Dnmt3-like (*Dnmt3l*) gene. *Dnmt3l* encodes a protein that shares homology with Dnmt3a and Dnmt3b in the PHD zinc-finger domain, but lacks the highly conserved methyltransferase motifs and has no enzymatic activity. Although *Dnmt3l*-deficient females produce mature and functional oocytes, embryos that are derived from these oocytes have neural-tube and placental abnormalities and die around midgestation[52,53]. The analysis of DNA-methylation patterns at several maternally methylated genes on different chromosomes, such as *Igf2r*, *Peg1* and *Peg3*, and of several imprinted genes in the *Snrpn* locus has shown that Dnmt3l-deficient oocytes fail to establish maternal-specific methylation imprints. Significantly, a failure to establish maternal methylation imprints in Dnmt3l-deficient oocytes results in the loss of the mono-allelic expression of all maternally imprinted genes that are examined in the offspring. These results show that methylation imprints that are acquired during oocyte maturation serve as the maternal genomic imprints.

Dnmt3l probably acts on imprinted genes through its interaction with the Dnmt3 family of DNA methyltransferases. Dnmt3l binds to and co-localizes with Dnmt3a and Dnmt3b in the nuclei of cells in which both of these proteins are expressed[53]. These results indicate that Dnmt3l might cooperate with Dnmt3a or Dnmt3b to regulate the gamete-specific methylation of imprinted genes in the oocyte (FIG. 2). Consistent with this model, it has been shown that *Dnmt3a*/*Dnmt3b*-deficient females also fail to establish maternal methylation imprints[53]. By contrast, inactivation of *Dnmt1* in the oocyte does not perturb the establishment of methylation imprints, but affects the maintenance of imprinting in pre-implantation embryos[54]. The possible involvement of histone modification and chromatin remodelling in the establishment of genomic imprints during gametogenesis remains to be investigated.

Methylation in spermatogenesis. In addition to its role in genomic imprinting in the female gamete, DNA methylation is also required for spermatogenesis. In *Dnmt3a*-knockout mice, the testes contain many abnormal spermatocytes at the meiosis prophase, but few mature sperms[53]. In the *Dnmt3l*-knockout mice, spermatogenesis is arrested at a time when the spermatogonia enter meiosis, which results in complete lack of mature sperm[52,53].

Interestingly, histone modification is also crucial in spermatogenesis. Mice in which both histone

GASTRULATION
A morphogenetic process that leads to the formation of the mesoderm layer between the endoderm and ectoderm layers and to the formation of embryonic body patterns.

DIFFERENTIALLY METHYLATED REGION
(DMR). DNA segments in imprinted genes that show different methylation patterns between paternal and maternal alleles. Some DMRs acquire DNA methylation in the germ cells, whereas others acquire DNA methylation during embryogenesis.

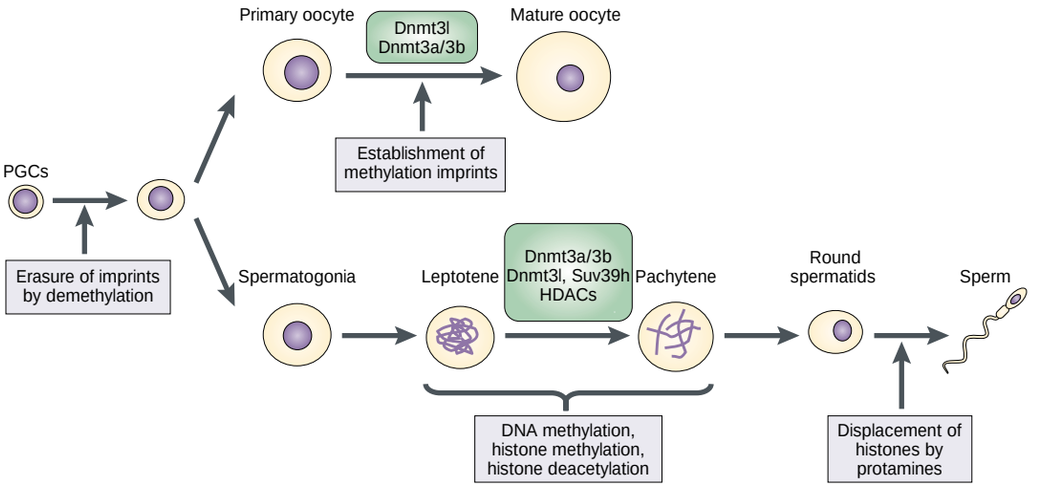

Figure 2 | **Epigenetic reprogramming during gametogenesis.** Primordial germ cells (PGCs) undergo demethylation at imprinted loci, which erases parental imprinting marks at around embryonic day 11.5–12.5 (REFS 50,51). The female PGCs develop to form primary oocytes. During oocyte growth and maturation, the maternal-specific genomic imprints are re-established through the *de novo* methylation activities of the methyltransferases Dnmt3a and Dnmt3b, and an associated protein Dnmt3l[52,53]. During spermatogenesis, several factors seem to function during the differentiation of the spermatocytes from the leptotene to pachytene stages of meiosis. During this period, histones are hypoacetylated, and the functions of Suv39h, Dnmt3a and Dnmt3l are essential[36,52,53,55]. The crucial stage when these factors function is not defined.

methyltransferases *Suv39h1* and *Suv39h2* have been knocked out are sterile[36]. In *Suv39h* double-knockout mice, homologous chromosome pairing is impaired, which results in meiotic arrest at the pachytene stage. The timing for the requirement of DNA methylation and histone H3-K9 methylation during spermatogenesis seems to correlate with histone deacetylation (FIG. 2). The core histones are hyperacetylated in spermatogonia and in pre-leptotene spermatocytes, but acetylated histones are not detected throughout meiosis in leptotene and pachytene spermatocytes, nor in most round spermatids[55]. These findings indicate that DNA methylation and histone modification might suppress global gene expression when spermatocytes are undergoing meiosis. Alternatively, these epigenetic changes might have a role in regulating chromosome architecture, which changes dynamically during spermatogenesis. It is of great importance to understand the specific chromosomal changes that require DNA methylation and histone modification during spermatogenesis and meiosis.

Epigenetic regulation of embryogenesis

Demethylation and remethylation. Both maternal and paternal genomes undergo rapid reprogramming after fertilization (FIG. 3). First, demethylation occurs in the male PRONUCLEUS, which seems to be independent of DNA replication[56,57]. After the formation of the zygote, both maternal and paternal chromosomes undergo progressive demethylation by a passive mechanism, which erases — by the BLASTOCYST stage — most, but not all, of the methylation marks that are inherited from the gametes[58–60]. The methylation marks on imprinted genes are, however, protected from demethylation, and therefore parental imprints are preserved. Whether extensive demethylation of the genome during pre-implantation development is essential for normal development is unknown. Embryonic DNA-methylation patterns are established after implantation through lineage-specific *de novo* methylation that begins in the INNER CELL MASS of a blastocyst[58,59,61] (FIG. 3). DNA-methylation levels increase rapidly in the primitive ectoderm, which gives rise to the entire embryo, whereas methylation is either inhibited or not maintained in the TROPHOBLAST and the PRIMITIVE ENDODERM lineage, which give rise to the placenta and YOLK-SAC membrane, respectively[62,63].

Genetic studies of the zygotic functions of DNA methyltransferases have shown that the establishment of embryonic methylation patterns requires both *de novo* and maintenance methyltransferase activities, and that the maintenance of genomic methylation above a threshold level is essential for embryonic development[6,7]. The inactivation of *Dnmt3a* and *Dnmt3b* blocks *de novo* methylation in early post-implantation mouse embryos and causes embryonic lethality, but has little effect on the maintenance of pre-existing methylation patterns[7]. *Dnmt1*-null mutant embryos arrest at late gastrulation stage and die around embryonic day (E)9.5 (REF. 64). The analysis of the methylation of certain DNA sequences, including repetitive elements (minor satellite repeats, endogenous C-type retrovirus and interstitial A particle (IAP) retrotransposons) and imprinted genes (*H19*, *Igf2r* and *Xist*), has shown that these sequences are all extensively demethylated[6,65–68] in *Dnmt1*-knockout embryos.

What role might such a dynamic demethylation and remethylation process have during early development? One preferred model is that genome-wide demethylation during pre-implantation development erases the methylation patterns (except those at imprinted genes) that are inherited from the gametes. This demethylation might lead to chromatin decondensation and to the

PRONUCLEUS
The sperm nucleus or the egg nucleus in a fertilized egg before a single nucleus forms.

BLASTOCYST
A pre-implantation embryo that contains a fluid-filled cavity called a blastocoel.

INNER CELL MASS
(ICM). A small clump of apparently undifferentiated cells in the blastocyst, which gives rise to the entire fetus and some of its extra-embryonic membranes.

TROPHOBLAST
The post-implantation derivatives of the trophectoderm, which make up most of the fetal part of the placenta.

PRIMITIVE ENDODERM
An early differentiated cell type that lines the inner surface of the blastocyst cavity. It gives rise to the endoderm component of the extra-embryonic membranes.

YOLK SAC
An extra-embryonic membrane that consists of an outer endoderm layer and an inner mesoderm layer, which surrounds the developing embryo.

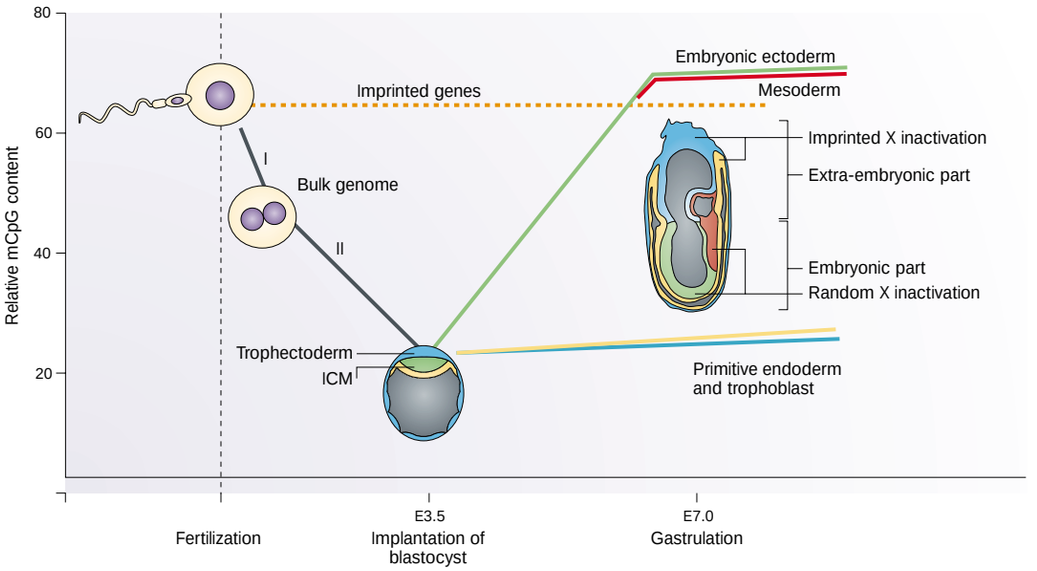

Figure 3 | **DNA-methylation reprogramming during early mouse development.** The methylation status of the bulk mouse genome, which consists of repeats and unique genes but excludes most CpG islands and imprinted regions, undergoes dynamic changes during early development[56–60]. After fertilization, the bulk genome undergoes demethylation through an active demethylation phase (I), followed by a passive demethylation phase (II). The methylation level of a blastocyst reaches the lowest point at embryonic day (E)3.5. After implantation, the bulk genome becomes hypermethylated in the embryonic ectoderm (green) and mesoderm (red) through active *de novo* methylation, whereas the genome of extra-embryonic cells, such as the primitive endoderm (yellow) and trophoblast (blue), remains hypomethylated. The parental methylation imprints in imprinted genes (orange) escape demethylation and *de novo* methylation. Interestingly, X inactivation is imprinted in the primitive endoderm (yellow) and the trophectoderm-derived cells (blue), whereas it is random in the embryonic tissues. ICM, inner cell mass.

transcriptional activation of the zygotic genes that are essential for early development; it might also facilitate subsequent genomic reprogramming through histone modification and chromatin remodelling. *De novo* methylation might cooperate with histone modification to repress retrotransposons and to establish a global silencing state. Such a model is consistent with the findings that many chromatin regulatory factors — including SWI/SNF family chromatin remodelling proteins, such as Brg1, transcription factors, such as YY1 and Ezh2, and DNA/histone-modification enzymes, such as HDAC1, the Dnmts and G9a — are required for embryonic development, from peri-implantation to late gastrulation (TABLE 1). The involvement of other chromatin regulators in genomic reprogramming during early development remains to be identified.

Maintaining DNA methylation. As mentioned above, maintaining DNA-methylation patterns in post-implantation embryos is essential for embryonic development. Dnmt1 maintains genomic methylation patterns in a DNA-replication-dependent manner. Complete inactivation of *Dnmt1* results in a 90% reduction of total methyl CpG in the genome after several rounds of DNA replication[64]. Interestingly, it has been shown that Dnmt3a and Dnmt3b are also required for the stable inheritance of DNA-methylation patterns in mouse embryonic stem (ES) cells. The inactivation of both *Dnmt3a* and *Dnmt3b* results in the rapid demethylation of the 5′ region of *Xist* and of the differentially methylated region Dmr2 of *Igf2*, and in a gradual demethylation of repetitive sequences[7]. A detailed analysis of a repeat sequence in *Dnmt1*- and in *Dnmt3*-knockout ES cells has provided evidence that Dnmt1 and Dnmt3 might cooperate to maintain DNA methylation after DNA replication[69]. In a human tumour cell line, HCT116, DNMT1 and DNMT3B have been shown to have overlapping functions in maintaining genome-wide methylation patterns[70]. Whether Dnmt3a and Dnmt3b are required for maintaining DNA-methylation patterns in somatic tissue remains to be determined.

For DNA methyltransferases to maintain dense methylation levels after DNA replication, the enzymes must pass efficiently through the nucleosomal DNA and methylate the cytosine bases that are embedded in the DNA helix. Proteins with chromatin-remodelling and DNA-helicase activities are therefore required to increase the accessibility of DNA to DNA methyltransferases. Indeed, two mammalian SNF2/helicase family proteins, Lsh and ATRX, can modulate DNA methylation in mammalian cells. In *Lsh*-knockout mice, repetitive DNA sequences, such as IAP, minor satellite, L1 and B1 repeats, are almost completely demethylated in E13.5 mutant embryos and in several tissues of newborn mice[26]. Some unique sequences, such as α-globin and the imprinted *H19* gene, are only partially demethylated in these mutants. Surprisingly, despite a severe reduction in DNA methylation, comparable with *Dnmt1*$^{-/-}$ embryos (particularly at repetitive DNA elements),

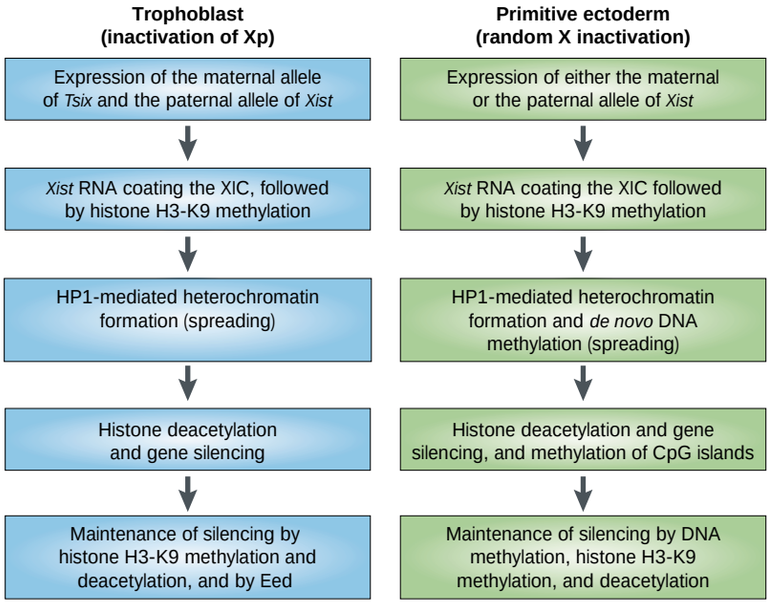

Figure 4 | **Regulation of X inactivation.** A comparison of imprinted X inactivation in the trophoblast (left) and random X inactivation in the primitive ectoderm (right), and the involvement of various factors. The role of HP1 in spreading and histone methylation in maintaining the inactive X remains to be tested. Xp, paternal X chromosome; XIC, X inactivation centre; H3-K9, lysine 9 of histone H3.

Lsh$^{-/-}$ mutant mice develop to term, although they die shortly after birth. This might be because embryonic methylation patterns are properly established in *Lsh*$^{-/-}$ embryos during early development, and therefore essential developmental processes, such as X inactivation, are uninterrupted.

ATRX contains a PHD-like zinc-finger domain, which is shared by the Dnmt3 protein family, and an SNF-like helicase domain. ATRX is associated with the nuclear matrix in interphase nuclei and with the acrocentric ribosomal DNA (rDNA) of metaphase chromosomes[71]. In humans, *ATRX* mutations cause a mental retardation syndrome, and some affected individuals show reduced expression of α-globin (which causes α-thalassaemia), hypomethylated rDNA and hypermethylated Y-chromosome repeats[25]. These findings indicate that ATRX, like Lsh, might modulate DNA methylation through its putative chromatin remodelling and DNA-helicase activity. The chromatin-remodelling/helicase activity that is required for *de novo* DNA methylation during early embryogenesis remains unknown.

Histone methylation (particularly at H3-K9) might also be required for the stable maintenance of DNA-methylation patterns during mammalian development, as such a mechanism has been shown to operate in both fungi and plants (as previously discussed). However, the identification of several families of histone H3-K9 methyltransferases, such as Suv39h1, Suv39h2, G9a, ESET and Eu-HMT1 in mammalian cells, indicates that different histone methyltransferases might be targeted to different regions of the genome to regulate chromatin structure and gene expression. Suv39h specifically methylates histone H3 in the pericentromeric hetero-chromatin[36], whereas G9a methylates histone H3-K9 in the euchromatin[37]. It will be interesting to examine whether Suv39 and G9a are required for DNA methylation in these two chromatin domains.

Maintaining genomic imprinting. Studies of *Dnmt1*-knockout mice have shown that DNA methylation is required for the maintenance of the mono-allelic expression of imprinted genes[65,67]. For example, in *Dnmt1*-deficient mouse embryos, alleles of both *Igf2* and *Igf2r*, which are normally paternally and maternally expressed, respectively, are silenced, whereas the *H19* gene, which is normally maternally expressed, is bi-allelically transcribed. This finding indicates that, in the absence of DNA methylation, other epigenetic mechanisms, such as histone modification, are either unstable or unable to maintain the mono-allelic expression of imprinted genes in developing mouse embryos. Other studies have shown that the transcribed alleles of imprinted genes, such as *H19*, *Snrpn* and *U2af1-rs1*, are associated with the hyperacetylation of histones H3 and H4, whereas the silent alleles of these genes are associated with hypoacetylation of the histones[72,73]. These findings indicate that the acetylation status of histones also contributes to the differential expression of the parental alleles of imprinted genes. Given the recent data from *Neurospora* and *Arabidopsis*, it is conceivable that histone methylation might also have a role in the maintenance of DNA methylation and genomic imprinting.

In summary, DNA methylation serves as an essential epigenetic mark for the establishment of genomic imprinting during germ-cell development, and it is also required for the maintenance of mono-allelic expression of imprinted genes during embryogenesis. The regulation of imprinted genes also involves histone acetylation and possibly histone methylation. At some imprinted loci, such as at *Igf2r*, *H19* and *Ube3a*, antisense RNAs have been identified. The expression of the antisense RNA (*Air*) of *Igf2r* has been shown to be required for the silencing of the paternal allele of *Igf2r*[48,74]. An important question is how these epigenetic mechanisms coordinate to establish and maintain the chromatin structures that differentiate paternal and maternal alleles of imprinted genes.

Regulating X inactivation

Dosage compensation in female mammals is achieved by X-chromosome inactivation, a process that silences one of the two X chromosomes during early embryogenesis. The X-inactivation process converts an X chromosome from active euchromatin into transcriptionally silent and highly condensed heterochromatin through a series of events that include the coating of the X chromosome by *Xist* RNA, DNA methylation and histone modification (FIG. 4). X inactivation provides a unique model for studying the establishment and maintenance of chromosome-wide gene silencing (see REF. 75 for more on the molecular mechanisms of X inactivation).

X inactivation occurs shortly after the implantation of female embryos, or on the induction of differentiation of female ES cells. It is accompanied by a marked increase in the expression and accumulation of *Xist* RNA. The *Xist* transcript then coats the inactive X chromosome, a step that is thought to be necessary and sufficient for the initiation of X inactivation. Targeted disruption of the *Xist* gene abrogates X inactivation[76,77]. Inducible expression of an *Xist* cDNA transgene in male ES cells is sufficient to trigger the inactivation of an autosome, in which the *Xist* transgene is integrated, which leads to the hypoacetylation of histone H4 and to the late replication of this chromosome[78]. However, the detailed mechanism by which *Xist* RNA orchestrates the assembly of an inactive X chromosome remains largely unknown.

In mice, X inactivation first occurs in the trophoblast cells of the placenta, and the paternal X chromosome is predominantly inactivated. The breakthrough in understanding this imprinted X inactivation in the trophoblast came with the discovery of *Tsix*, an antisense RNA of *Xist*, which, like *Xist*, is a non-coding RNA[79]. The *Tsix* transcript is first detected in the blastocyst, and only the maternal allele of *Tsix* is expressed, although it is not clear whether *Tsix* expression is restricted to the TROPHECTODERM cells at this stage. *Tsix* has been shown to repress *Xist* expression *in cis*[80–82], possibly by an RNA-interference mechanism that destabilizes *Xist* RNA or by silencing the *Xist* promoter. In mice, the deletion of the main *Tsix* promoter, or the disruption of *Tsix* transcripts from the maternal allele of *Tsix*, results in the expression of maternal *Xist*. It also leads to the inactivation of both paternal and maternal X chromosomes in the trophoblast cells, which results in embryonic lethality[81,83]. These experiments show that the expression of maternal *Tsix* at the blastocyst stage leads to the exclusive expression of the paternal allele of *Xist* and to the inactivation of the paternal X chromosome in the trophoblast cells of the placenta. The choice of which X chromosome to inactivate is random in the primitive ectoderm lineage, which gives rise to the embryo proper. Random X inactivation in embryonic tissues is probably caused by the erasure of an imprinting mark that controls the imprinted expression of *Xist* and *Tsix*. Whether DNA methylation is the imprinting mark that is erased in the embryonic lineage to allow random X inactivation remains to be determined. Interestingly, the deletion of *Tsix* results in non-random X inactivation in embryonic tissues, indicating that random *Tsix* expression in embryonic cells contributes to the random expression of the two *Xist* alleles[81,83].

One important feature of X inactivation is its spreading from the X inactivation centre (XIC), where this process is initiated, to the entire X chromosome. This leads to chromosome-wide transcriptional silencing, condensation and the late replication of the inactive X. Finding the molecules that are involved in this process is the goal of an intensive research effort. A recent study of full-length *Xist* cDNA and of various deleted forms of *Xist* identified that the 5′ region of *Xist* is required for long-range silencing on the inactive X, whereas several other regions of the *Xist* RNA are required for *Xist* to coat the X chromosome[84]. Similarly, the use of an antisense oligonucleotide against the 5′ region of *Xist* transcripts has been shown to disrupt X inactivation[85].

Recent studies of histone methylation have shown that the differential methylation of H3-K4 and H3-K9 also has a crucial role in X inactivation. Methylation of histone H3-K9 is associated with the inactive X, whereas methylation of H3-K4 is associated with the active X[86,87]. Of special interest is the finding that methylation of histone H3-K9 occurs first in the 5′ region of *Xist*, immediately after *Xist* RNA has coated the X chromosome to be inactivated, but before the transcriptional silencing of X-linked genes occurs[87]. These observations support a model in which *Xist* RNA recruits both histone H3-K9 methyltransferases and histone deacetylases to the inactive X by coating it, and then directs the spreading of inactivation along the X chromosome through heterochromatin formation[87].

The involvement of other factors, such as those that bring about DNA methylation in the initiation and spreading of X inactivation, remains to be investigated. Studies of *Dnmt1*-knockout mice indicate that the zygotic function of Dnmt1 might not be essential for the establishment of X inactivation in embryonic and extra-embryonic tissues[88]. However, the presence of maternal Dnmt1, Dnmt3a and Dnmt3b might be sufficient to allow the initiation and spreading of X inactivation to proceed. *De novo* methylation of CpG islands and repeats on the inactive X by the Dnmt3a and Dnmt3b enzymes might facilitate the spreading of the inactive signal or might stabilize the inactivated portion of the X. Hypermethylation might recruit a methyl-CpG-binding protein, such as Mecp2 and Mbd2, which in turn would recruit chromatin-remodelling and histone-deacetylase complexes. It will be interesting to test whether X inactivation is affected in *Dnmt3a* and *Dnmt3b* double-mutant mouse embryos and to determine which MBD and chromatin-remodelling complexes are involved in X inactivation.

The maintenance of the inactive X and the transcriptional silencing of X-linked genes involve several factors. Although *Xist* RNA is not essential for the maintenance of the inactive X in cultured human and mouse cells[89,90], DNA methylation and histone deacetylation are crucial for the stable silencing of the inactive X chromosome in somatic cells. In *Dnmt1*-knockout embryos, an X-linked transgene is reactivated when DNA-methylation levels decrease below a certain threshold in *Dnmt1*-knockout embryos[88]. In human ICF (immunodeficiency, centromeric instability and facial anomaly) patients, who have *DNMT3B* mutations, some genes on the inactive X are hypomethylated and reactivated[91]. It is also well documented that the inactive X is hypoacetylated and the active X is hyperacetylated[92]. Studies that used mouse embryonic fibroblast cells have shown that the maintenance of stable X inactivation requires the synergistic actions of *Xist* RNA, DNA methylation and histone deacetylation[93]. Histone H3-K9 methylation is probably also required for the propagation of heterochromatin

TROPHECTODERM
The outer epithelial layer of the blastocyst.

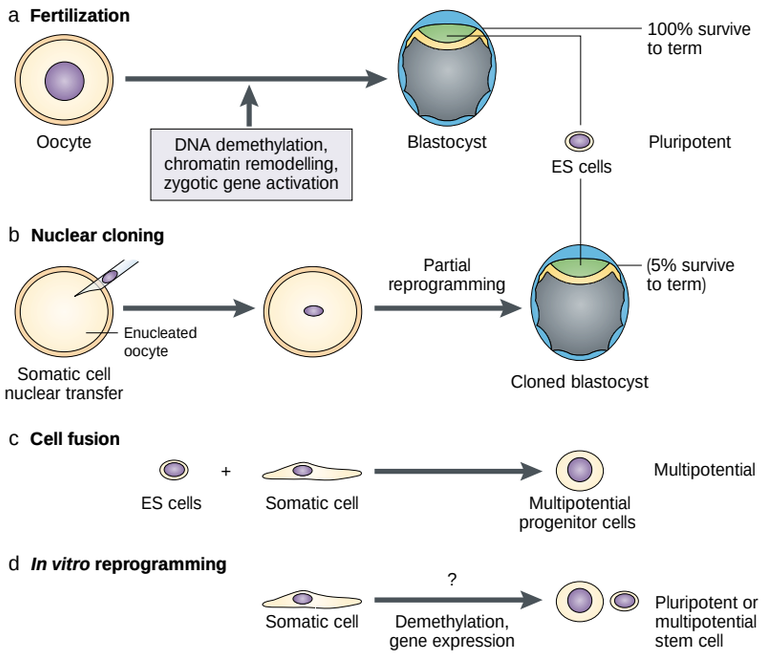

Figure 5 | **Nuclear reprogramming. a** | A normally fertilized egg is a totipotent cell that gives rise to the entire embryo and to the extra-embryonic tissues. Embryonic stem (ES) cells that are derived from the inner cell mass of a blastocyst can also give rise to the entire embryo proper, but not to the extra-embryonic tissues. **b** | Nuclei of somatic cells can be reprogrammed in enucleated oocytes. However, only 5% of cloned embryos develop to term. Pluripotent ES cells can also be derived from cloned blastocysts. **c** | Multipotential progenitor cells can be derived by fusing ES cells with somatic cells, such as neurons and lymphocytes. **d** | *In vitro* reprogramming of somatic nuclei to convert somatic cells into pluripotent stem cells has yet to be achieved.

and for the stable gene silencing that is associated with the inactive X (FIG. 4). One recent study has shown that a Polycomb group protein, Eed, is essential for the stable silencing of an X-linked GFP (green fluorescent protein) transgene in trophoblast cells[94]. Together, these findings indicate that chromosome-wide gene silencing requires the synergistic action of several epigenetic mechanisms.

Reprogramming the somatic nucleus

Nuclear cloning experiments in mammals have shown that a somatic cell nucleus from adult tissue can initiate embryonic development after being transplanted into an enucleated oocyte[95,96] (FIG. 5). The remarkable ability of the cytoplasm of an oocyte to trigger the 'de-differentiation' of an adult nucleus indicates that a committed or differentiated cell fate can be reversed through epigenetic reprogramming. However, cloning is inefficient, as most cloned embryos die shortly after implantation and the few that survive to birth frequently have developmental abnormalities and seem to have a short lifespan[97–99]. This indicates that the reprogramming of the transplanted nucleus is incomplete, as compared with that in a fertilized egg from natural mating.

What processes are not reprogrammed properly in cloned embryos, leading to the high incidence of developmental arrest among them? Apparently, telomere length, which shortens with time in somatic cells, can be restored in cloned bovine embryos[100]. Studies of methylation have shown that genome-wide demethylation before implantation is less efficient in cloned bovine embryos, and that *de novo* methylation occurs at an earlier stage in cloned embryos compared with normal embryos[101–104]. The abnormal reprogramming of DNA methylation could result in the failed reactivation of the genes that are essential for embryonic development. It has been shown that, in cloned mouse blastocysts, expression of *Oct4* is reduced in the inner cell mass and is ectopic in trophoblast cells[105]. Because Oct4 is a transcription factor that is specifically expressed in stem cells and primordial germ cells, and is required for maintaining PLURIPOTENCY and the self-renewal ability of the stem cell[106], its aberrant expression in cloned embryos could lead to the abnormal expression of other downstream genes that are required for early embryogenesis. The analysis of imprinted genes in cloned mouse embryos has also shown that, although their parental-specific expression patterns are faithfully maintained in cloned embryos, their expression levels are below normal in the placenta[107]. Aberrant expression of imprinted genes and of *Oct4* might contribute to the placental defects that frequently occur in cloned animals[97,108]. Further studies are necessary to determine whether histone modification and the expression of chromatin-remodelling proteins are normal in cloned embryos.

It is also remarkable that pluripotent ES cells can be isolated from cloned embryos that have been derived from terminally differentiated nuclei[109]. This experiment indicates that epigenetic reprogramming of transplanted somatic nuclei in oocytes and in pre-implantation embryos is sufficient to convert a terminally differentiated adult cell into a pluripotent stem cell. In mice, it has been shown that gene targeting in ES cells, derived from cloned embryos that harbour genetic mutations, can be used to correct such mutations. The cells can then be used to generate normal tissues and healthy mice[110]. This procedure is believed to be potentially useful for therapeutic purposes in humans. Can terminally differentiated cells, such as a skin cell, be reprogrammed *in vitro* to give rise to pluripotent stem cells? If the de-differentiation process of somatic nuclei in cloned embryos could be recapitulated *in vitro*, we could bypass the complicated cloning procedure and produce pluripotent or multipotential stem cells from unlimited adult tissues. Partial reprogramming of somatic nuclei can be achieved *in vitro* by fusing somatic cells with ES cells[111,112]. A current controversy is whether tissue-specific stem cells that are isolated from adult tissues transdifferentiate into other tissues or whether they acquire new abilities by fusing with differentiated cells[112,113]. Although there is no direct evidence that fusion occurs *in vivo*, it has been shown that a single mesenchymal stem cell that has been isolated from adult marrow can contribute to several tissues after being injected into a blastocyst[113]. Studies of the molecular mechanisms of nuclear reprogramming at the level of DNA and chromatin might make it possible to convert differentiated somatic cells to pluripotent stem cells *in vitro*.

PLURIPOTENCY
The ability of a cell to contribute to several tissues in a developing organism. If a cell is able to contribute to all tissues, it is said to be totipotent.

Conclusions

Epigenetic reprogramming of the genome by DNA methylation, histone modification and chromatin remodelling during gametogenesis and early embryogenesis sets the developmental programme for normal embryogenesis. Studies of developmental programmes, such as genomic imprinting and X inactivation, in normal and cloned animals have begun to unravel how epigenetic reprogramming works. The recent discovery of many DNA- and histone-modification enzymes, and of chromatin-remodelling and transcription-regulatory complexes, is expected to accelerate our understanding of the epigenetic mechanisms that control mammalian development. The challenge that we are facing is not so much to understand how individual molecules might function, but to assemble different proteins into regulatory complexes and pathways; in particular, to elucidate how these protein complexes are targeted to specific genes or chromosomal domains. Alterations to DNA methylation or chromatin structure have been linked to various human diseases, such as mental retardation syndromes and cancer (see REF. 114 for more on the role of epigenetics in cancer). Studies of the epigenetic regulation of chromatin structure and gene expression in development will help to elucidate the underlying cause of such diseases. A greater understanding of these processes is also of clinical importance. Although pluripotent stem cells or progenitor cells can be used in cell-replacement therapy to treat degenerative diseases, new drugs that target epigenetic regulators can be developed to treat developmental disorders or cancers that are caused by altered epigenetic states.

1. Bird, A. DNA methylation patterns and epigenetic memory. *Genes Dev*. **16**, 6–21 (2002).
2. Jenuwein, T. & Allis, C. D. Translating the histone code. *Science* **293**, 1074–1080 (2001).
3. Zhang, Y. & Reinberg, D. Transcription regulation by histone methylation: interplay between different covalent modifications of the core histone tails. *Genes Dev*. **15**, 2343–2360 (2001).
4. Narlikar, G. J., Fan, H. Y. & Kingston, R. E. Cooperation between complexes that regulate chromatin structure and transcription. *Cell* **108**, 475–487 (2002).
5. Bestor, T. H. Activation of mammalian DNA methyltransferase by cleavage of a Zn binding regulatory domain. *EMBO J.* **11**, 2611–2617 (1992).
6. Li, E., Bestor, T. H. & Jaenisch, R. Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. *Cell* **69**, 915–926 (1992).
7. Okano, M., Bell, D. W., Haber, D. A. & Li, E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for *de novo* methylation and mammalian development. *Cell* **99**, 247–257 (1999).
   This paper provided the first genetic evidence that the Dnmt3 family of methyltransferases is responsible for *de novo* methylation in mouse ES cells and during development.
8. Lyko, F. *et al.* Mammalian (cytosine-5) methyltransferases cause genomic DNA methylation and lethality in *Drosophila*. *Nature Genet.* **23**, 363–366 (1999).
9. Hsieh, C. L. *In vivo* activity of murine *de novo* methyltransferases, Dnmt3a and Dnmt3b. *Mol. Cell. Biol.* **19**, 8211–8218 (1999).
10. Burgers, W. A., Fuks, F. & Kouzarides, T. DNA methyltransferases get connected to chromatin. *Trends Genet.* **18**, 275–277 (2002).
11. Takizawa, T. *et al.* DNA methylation is a critical cell-intrinsic determinant of astrocyte differentiation in the fetal brain. *Dev. Cell* **1**, 749–758 (2001).
    This paper shows that the demethylation of a promoter element induces tissue-specific gene expression during cellular differentiation.
12. Nan, X. *et al.* Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. *Nature* **393**, 386–389 (1998).
13. Jones, P. L. *et al.* Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. *Nature Genet.* **19**, 187–191 (1998).
    References 12 and 13 provided the first molecular evidence linking DNA methylation to histone deacetylation in transcription repression.
14. Zhang, Y. *et al.* Analysis of the NuRD subunits reveals a histone deacetylase core complex and a connection with DNA methylation. *Genes Dev*. **13**, 1924–1935 (1999).
15. Wade, P. A. *et al.* Mi-2 complex couples DNA methylation to chromatin remodelling and histone deacetylation. *Nature Genet.* **23**, 62–66 (1999).
16. Ng, H. H. *et al.* MBD2 is a transcriptional repressor belonging to the MeCP1 histone deacetylase complex. *Nature Genet*. **23**, 58–61 (1999).
17. Feng, Q. & Zhang, Y. The MeCP1 complex represses transcription through preferential binding, remodeling, and deacetylating methylated nucleosomes. *Genes Dev*. **15**, 827–832 (2001).
    This study shows how MBD2 might recruit the NuRD remodelling complex to methylated chromatin to remodel the chromatin and to silence transcription.
18. Murrell, A. *et al.* An intragenic methylated region in the imprinted *Igf2* gene augments transcription. *EMBO Rep.* **2**, 1101–1106 (2001).
19. Eden, S. *et al.* An upstream repressor element plays a role in *Igf2* imprinting. *EMBO J.* **20**, 3518–3525 (2001).
20. Strahl, B. D. & Allis, C. D. The language of covalent histone modifications. *Nature* **403**, 41–45 (2000).
21. Tamaru, H. & Selker, E. U. A histone H3 methyltransferase controls DNA methylation in *Neurospora crassa*. *Nature* **414**, 277–283 (2001).
22. Jackson, J. P., Lindroth, A. M., Cao, X. & Jacobsen, S. E. Control of CpNpG DNA methylation by the KRYPTONITE histone H3 methyltransferase. *Nature* **416**, 556–560 (2002).
    References 21 and 22 provide molecular and genetic evidence that histone H3-K9 methylation is required for DNA methylation.
23. Jeddeloh, J. A., Stokes, T. L. & Richards, E. J. Maintenance of genomic methylation requires a SWI2/SNF2-like protein. *Nature Genet.* **22**, 94–97 (1999).
24. Gendrel, A. V., Lippman, Z., Yordan, C., Colot, V. & Martienssen, R. Dependence of heterochromatic histone H3 methylation patterns on the *Arabidopsis* gene *DDM1*. *Science* **20** (in the press).
25. Gibbons, R. J. *et al.* Mutations in *ATRX*, encoding a SWI/SNF-like protein, cause diverse changes in the pattern of DNA methylation. *Nature Genet.* **24**, 368–371 (2000).
26. Dennis, K., Fan, T., Geiman, T., Yan, Q. & Muegge, K. Lsh, a member of the SNF2 family, is required for genome-wide methylation. *Genes Dev*. **15**, 2940–2944 (2001).
    References 23–26 show that SWI/SNF-like chromatin-remodelling proteins are intimately involved in regulating DNA methylation and histone methylation.
27. Bultman, S. *et al.* A *Brg1* null mutation in the mouse reveals functional differences among mammalian SWI/SNF complexes. *Mol. Cell* **6**, 1287–1295 (2000).
28. Hendrich, B., Guy, J., Ramsahoye, B., Wilson, V. A. & Bird, A. Closely related proteins MBD2 and MBD3 play distinctive but interacting roles in mouse development. *Genes Dev*. **15**, 710–723 (2001).
29. O'Carroll, D. *et al.* The polycomb-group gene *Ezh2* is required for early mouse development. *Mol. Cell. Biol.* **21**, 4330–4336 (2001).
30. Donohoe, M. E. *et al.* Targeted disruption of mouse Yin Yang 1 transcription factor results in peri-implantation lethality. *Mol. Cell. Biol.* **19**, 7237–7244 (1999).
31. Faust, C., Lawson, K. A., Schork, N. J., Thiel, B. & Magnuson, T. The Polycomb-group gene *eed* is required for normal morphogenetic movements during gastrulation in the mouse embryo. *Development* **125**, 4495–4506 (1998).
32. Lagger, G. *et al.* Essential function of histone deacetylase 1 in proliferation control and CDK inhibitor repression. *EMBO J.* **21**, 2672–2681 (2002).
    This paper provides genetic evidence that histone deacetylation is essential for embryonic development.
33. Guy, J., Hendrich, B., Holmes, M., Martin, J. E. & Bird, A. A mouse *Mecp2*-null mutation causes neurological symptoms that mimic Rett syndrome. *Nature Genet.* **27**, 322–326 (2001).
34. Chen, R. Z., Akbarian, S., Tudor, M. & Jaenisch, R. Deficiency of methyl-CpG binding protein-2 in CNS neurons results in a Rett-like phenotype in mice. *Nature Genet.* **27**, 327–331 (2001).
35. Shahbazian, M. D. *et al.* Mice with truncated MeCP2 recapitulate many Rett syndrome features and display hyperacetylation of histone H3. *Neuron* **35**, 243–254 (2002).
36. Peters, A. H. *et al.* Loss of the Suv39h histone methyltransferases impairs mammalian heterochromatin and genome stability. *Cell* **107**, 323–337 (2001).
37. Tachibana, M. *et al.* G9a histone methyltransferase plays a dominant role in euchromatic histone H3 lysine 9 methylation and is essential for early embryogenesis. *Genes Dev*. **16**, 1779–1791 (2002).
    References 36 and 37 show that two histone H3-K9 methyltransferases have distinct functions in mouse development. Suv39h methylates histones in heterochromatin and is dispensable for early development, whereas G9a methylates histones in euchromatin and is essential for early development.
38. Lachner, M., O'Carroll, D., Rea, S., Mechtler, K. & Jenuwein, T. Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. *Nature* **410**, 116–120 (2001).
39. Bannister, A. J. *et al.* Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. *Nature* **410**, 120–124 (2001).
40. Jones, D. O., Cowell, I. G. & Singh, P. B. Mammalian chromodomain proteins: their role in genome organisation and expression. *Bioessays* **22**, 124–137 (2000).
41. Surani, M. A., Barton, S. C. & Norris, M. L. Development of reconstituted mouse eggs suggests imprinting of the genome during gametogenesis. *Nature* **308**, 548–550 (1984).
42. McGrath, J. & Solter, D. Completion of mouse embryogenesis requires both the maternal and paternal genomes. *Cell* **37**, 179–183 (1984).
43. Reik, W. & Walter, J. Genomic imprinting: parental influence on the genome. *Nature Rev. Genet.* **2**, 21–32 (2001).
44. Tremblay, K. D., Saam, J. R., Ingram, R. S., Tilghman, S. M. & Bartolomei, M. S. A paternal-specific methylation imprint marks the alleles of the mouse *H19* gene. *Nature Genet.* **9**, 407–413 (1995).
45. Thorvaldsen, J. L., Duran, K. L. & Bartolomei, M. S. Deletion of the H19 differentially methylated domain results in loss of imprinted expression of *H19* and *Igf2*. *Genes Dev*. **12**, 3693–3702 (1998).
46. Yoon, B. J. *et al.* Regulation of DNA methylation of Rasgrf1. *Nature Genet.* **30**, 92–96 (2002).
47. Stoger, R. *et al.* Maternal-specific methylation of the imprinted mouse *Igf2r* locus identifies the expressed locus as carrying the imprinting signal. *Cell* **73**, 61–71 (1993).
48. Wutz, A. *et al.* Imprinted expression of the *Igf2r* gene depends on an intronic CpG island. *Nature* **389**, 745–749 (1997).
49. Shemer, R., Birger, Y., Riggs, A. D. & Razin, A. Structure of the imprinted mouse *Snrpn* gene and establishment of its parental-specific methylation pattern. *Proc. Natl Acad. Sci. USA* **94**, 10267–10272 (1997).
50. Lee, J. *et al.* Erasing genomic imprinting memory in mouse clone embryos produced from day 11.5 primordial germ cells. *Development* **129**, 1807–1817 (2002).
51. Hajkova, P. *et al.* Epigenetic reprogramming in mouse primordial germ cells. *Mech. Dev.* (in the press).
    References 50 and 51 defined the embryonic stage when imprinting marks are erased in the germ cells.

52. Bourc'his, D., Xu, G. L., Lin, C. S., Bollman, B. & Bestor, T. H. Dnmt3L and the establishment of maternal genomic imprints. *Science* **294**, 2536–2539 (2001).
53. Hata, K., Okano, M., Lei, H. & Li, E. Dnmt3L cooperates with the Dnmt3 family of *de novo* DNA methyltransferases to establish maternal imprints in mice. *Development* **129**, 1983–1993 (2002).
54. Howell, C. Y. *et al.* Genomic imprinting disrupted by a maternal effect mutation in the *Dnmt1* gene. *Cell* **104**, 829–838 (2001).
References 52–54 provide genetic evidence that Dnmt3a, Dnmt3b and Dnmt3l but not Dnmt1 are responsible for establishing the imprinting marks through *de novo* methylation in oocytes.
55. Hazzouri, M. *et al.* Regulated hyperacetylation of core histones during mouse spermatogenesis: involvement of histone deacetylases. *Eur. J. Cell Biol.* **79**, 950–960 (2000).
56. Mayer, W., Niveleau, A., Walter, J., Fundele, R. & Haaf, T. Demethylation of the zygotic paternal genome. *Nature* **403**, 501–502 (2000).
57. Oswald, J. *et al.* Active demethylation of the paternal genome in the mouse zygote. *Curr. Biol.* **10**, 475–478 (2000).
58. Howlett, S. K. & Reik, W. Methylation levels of maternal and paternal genomes during preimplantation development. *Development* **113**, 119–127 (1991).
59. Kafri, T. *et al.* Developmental pattern of gene-specific DNA methylation in the mouse embryo and germ line. *Genes Dev.* **6**, 705–714 (1992).
60. Rougier, N. *et al.* Chromosome methylation patterns during mammalian preimplantation development. *Genes Dev*. **12**, 2108–2113 (1998).
61. Santos, F., Hendrich, B., Reik, W. & Dean, W. Dynamic reprogramming of DNA methylation in the early mouse embryo. *Dev. Biol.* **241**, 172–182 (2002).
62. Chapman, V., Forrester, L., Sanford, J., Hastie, N. & Rossant, J. Cell lineage-specific undermethylation of mouse repetitive DNA. *Nature* **307**, 284–286 (1984).
63. Rossant, J., Sanford, J. P., Chapman, V. M. & Andrews, G. K. Undermethylation of structural gene sequences in extraembryonic lineages of the mouse. *Dev. Biol.* **117**, 567–573 (1986).
64. Lei, H. *et al. De novo* DNA cytosine methyltransferase activities in mouse embryonic stem cells. *Development* **122**, 3195–3205 (1996).
65. Li, E., Beard, C. & Jaenisch, R. Role for DNA methylation in genomic imprinting. *Nature* **366**, 362–365 (1993).
66. Beard, C., Li, E. & Jaenisch, R. Loss of methylation activates *Xist* in somatic but not in embryonic cells. *Genes Dev.* **9**, 2325–2334 (1995).
67. Caspary, T., Cleary, M. A., Baker, C. C., Guan, X. J. & Tilghman, S. M. Multiple mechanisms regulate imprinting of the mouse distal chromosome 7 gene cluster. *Mol. Cell. Biol.* **18**, 3466–3474 (1998).
68. Walsh, C. P., Chaillet, J. R. & Bestor, T. H. Transcription of IAP endogenous retroviruses is constrained by cytosine methylation. *Nature Genet.* **20**, 116–117 (1998).
69. Liang, G. *et al.* Cooperativity between DNA methyltransferases in the maintenance methylation of repetitive elements. *Mol. Cell. Biol.* **22**, 480–491 (2002).
70. Rhee, I. *et al.* DNMT1 and DNMT3b cooperate to silence genes in human cancer cells. *Nature* **416**, 552–556 (2002).
71. McDowell, T. L. *et al.* Localization of a putative transcriptional regulator (ATRX) at pericentromeric heterochromatin and the short arms of acrocentric chromosomes. *Proc. Natl Acad. Sci. USA* **96**, 13983–13988 (1999).
72. Gregory, R. I. *et al.* DNA methylation is linked to deacetylation of histone H3, but not H4, on the imprinted genes *Snrpn* and *U2af1-rs1*. *Mol. Cell. Biol.* **21**, 5426–5436 (2001).
73. Grandjean, V., O'Neill, L., Sado, T., Turner, B. & Ferguson-Smith, A. Relationship between DNA methylation, histone H4 acetylation and gene expression in the mouse imprinted Igf2-H19 domain. *FEBS Lett.* **488**, 165–169 (2001).
74. Sleutels, F., Zwart, R. & Barlow, D. P. The non-coding *Air* RNA is required for silencing autosomal imprinted genes. *Nature* **415**, 810–813 (2002).
This study shows that a non-coding RNA is involved in regulating genomic imprinting.
75. Avner, P. & Heard, E. X-chromosome inactivation: counting, choice and initiation. *Nature Rev. Genet.* **2**, 59–67 (2001).
76. Penny, G. D., Kay, G. F., Sheardown, S. A., Rastan, S. & Brockdorff, N. Requirement for Xist in X chromosome inactivation. *Nature* **379**, 131–137 (1996).
77. Marahrens, Y., Panning, B., Dausman, J., Strauss, W. & Jaenisch, R. *Xist*-deficient mice are defective in dosage compensation but not spermatogenesis. *Genes Dev*. **11**, 156–166 (1997).
78. Wutz, A. & Jaenisch, R. A shift from reversible to irreversible X inactivation is triggered during ES cell differentiation. *Mol. Cell* **5**, 695–705 (2000).
79. Lee, J. T., Davidow, L. S. & Warshawsky, D. *Tsix*, a gene antisense to Xist at the X-inactivation centre. *Nature Genet.* **21**, 400–404 (1999).
80. Luikenhuis, S., Wutz, A. & Jaenisch, R. Antisense transcription through the *Xist* locus mediates Tsix function in embryonic stem cells. *Mol. Cell. Biol.* **21**, 8512–8520 (2001).
81. Sado, T., Wang, Z., Sasaki, H. & Li, E. Regulation of imprinted X-chromosome inactivation in mice by *Tsix*. *Development* **128**, 1275–1286 (2001).
82. Stavropoulos, N., Lu, N. & Lee, J. T. A functional role for Tsix transcription in blocking *Xist* RNA accumulation but not in X-chromosome choice. *Proc. Natl Acad. Sci. USA* **98**, 10232–10237 (2001).
83. Lee, J. T. Disruption of imprinted X inactivation by parent-of-origin effects at *Tsix*. *Cell* **103**, 17–27 (2000).
84. Wutz, A., Rasmussen, T. P. & Jaenisch, R. Chromosomal silencing and localization are mediated by different domains of *Xist* RNA. *Nature Genet.* **30**, 167–174 (2002).
85. Beletskii, A., Hong, Y. K., Pehrson, J., Egholm, M. & Strauss, W. M. PNA interference mapping demonstrates functional domains in the noncoding RNA *Xist*. *Proc. Natl Acad. Sci. USA* **98**, 9215–9220 (2001).
86. Boggs, B. A. *et al.* Differentially methylated forms of histone H3 show unique association patterns with inactive human X chromosomes. *Nature Genet.* **30**, 73–76 (2002).
87. Heard, E. *et al.* Methylation of histone H3 at Lys-9 is an early mark on the X chromosome during X inactivation. *Cell* **107**, 727–738 (2001).
References 86 and 87 show that H3-K9 methylation correlates with the inactive X, whereas H3-K4 methylation correlates with the active X. H3-K9 methylation is also an early event in the initiation of X inactivation.
88. Sado, T. *et al.* X inactivation in the mouse embryo deficient for *Dnmt1*: distinct effect of hypomethylation on imprinted and random X inactivation. *Dev. Biol.* **225**, 294–303 (2000).
89. Brown, C. J. & Willard, H. F. The human X-inactivation centre is not required for maintenance of X-chromosome inactivation. *Nature* **368**, 154–156 (1994).
90. Csankovszki, G., Panning, B., Bates, B., Pehrson, J. R. & Jaenisch, R. Conditional deletion of *Xist* disrupts histone macroH2A localization but not maintenance of X inactivation. *Nature Genet.* **22**, 323–324 (1999).
91. Hansen, R. S. *et al.* Escape from gene silencing in ICF syndrome: evidence for advanced replication time as a major determinant. *Hum. Mol. Genet.* **9**, 2575–2587 (2000).
92. Jeppesen, P. & Turner, B. M. The inactive X chromosome in female mammals is distinguished by a lack of histone H4 acetylation, a cytogenetic marker for gene expression. *Cell* **74**, 281–289 (1993).
93. Csankovszki, G., Nagy, A. & Jaenisch, R. Synergism of *Xist* RNA, DNA methylation, and histone hypoacetylation in maintaining X chromosome inactivation. *J. Cell Biol.* **153**, 773–784 (2001).
94. Wang, J. *et al.* Imprinted X inactivation maintained by a mouse Polycomb group gene. *Nature Genet.* **28**, 371–375 (2001).
This work shows that a Polycomb group gene is required for maintaining imprinted X inactivation.
95. Wilmut, I., Schnieke, A. E., McWhir, J., Kind, A. J. & Campbell, K. H. Viable offspring derived from fetal and adult mammalian cells. *Nature* **385**, 810–813 (1997).
96. Wakayama, T., Perry, A. C., Zuccotti, M., Johnson, K. R. & Yanagimachi, R. Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. *Nature* **394**, 369–374 (1998).
97. Tanaka, S. *et al.* Placentomegaly in cloned mouse concepti caused by expansion of the spongiotrophoblast layer. *Biol. Reprod.* **65**, 1813–1821 (2001).
98. Tamashiro, K. L. *et al.* Cloned mice have an obese phenotype not transmitted to their offspring. *Nature Med.* **8**, 262–267 (2002).
99. Ogonuki, N. *et al.* Early death of mice cloned from somatic cells. *Nature Genet.* **30**, 253–254 (2002).
100. Tian, X. C., Xu, J. & Yang, X. Normal telomere lengths found in cloned cattle. *Nature Genet.* **26**, 272–273 (2000).
101. Kang, Y. K. *et al.* Aberrant methylation of donor genome in cloned bovine embryos. *Nature Genet.* **28**, 173–177 (2001).
102. Dean, W. *et al.* Conservation of methylation reprogramming in mammalian development: aberrant reprogramming in cloned embryos. *Proc. Natl Acad. Sci. USA* **98**, 13734–13738 (2001).
103. Bourc'his, D. *et al.* Delayed and incomplete reprogramming of chromosome methylation patterns in bovine cloned embryos. *Curr. Biol.* **11**, 1542–1546 (2001).
104. Kang, Y. K. *et al.* Limited demethylation leaves mosaic-type methylation states in cloned bovine pre-implantation embryos. *EMBO J.* **21**, 1092–1100 (2002).
105. Boiani, M., Eckardt, S., Scholer, H. R. & McLaughlin, K. J. Oct4 distribution and level in mouse clones: consequences for pluripotency. *Genes Dev.* **16**, 1209–1219 (2002).
106. Niwa, H., Miyazaki, J. & Smith, A. G. Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. *Nature Genet.* **24**, 372–376 (2000).
107. Inoue, K. *et al.* Faithful expression of imprinted genes in cloned mice. *Science* **295**, 297 (2002).
108. Rideout, W. M. III, Eggan, K. & Jaenisch, R. Nuclear cloning and epigenetic reprogramming of the genome. *Science* **293**, 1093–1098 (2001).
109. Hochedlinger, K. & Jaenisch, R. Monoclonal mice generated by nuclear transfer from mature B and T donor cells. *Nature* **415**, 1035–1038 (2002).
110. Rideout, W. M. III, Hochedlinger, K., Kyba, M., Daley, G. Q. & Jaenisch, R. Correction of a genetic defect by nuclear transplantation and combined cell and gene therapy. *Cell* **109**, 17–27 (2002).
This work shows the feasibility of therapeutic cloning in a mouse model.
111. Tada, M., Takahama, Y., Abe, K., Nakatsuji, N. & Tada, T. Nuclear reprogramming of somatic cells by *in vitro* hybridization with ES cells. *Curr. Biol.* **11**, 1553–1558 (2001).
112. Ying, Q. L., Nichols, J., Evans, E. P. & Smith, A. G. Changing potency by spontaneous fusion. *Nature* **416**, 545–548 (2002).
113. Jiang, Y. *et al.* Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* **418**, 41–49 (2002).
114. Jones, P. A. & Baylin, S. B. The fundamental role of epigenetic events in cancer. *Nature Rev. Genet.* **3**, 415–428 (2002).
115. Okano, M., Xie, S. & Li, E. Dnmt2 is not required for *de novo* and maintenance methylation of viral DNA in embryonic stem cells. *Nucleic Acids Res.* **26**, 2536–2540 (1998).
116. Hendrich, B., Hardeland, U., Ng, H. H., Jiricny, J. & Bird, A. The thymine glycosylase MBD4 can bind to the product of deamination at methylated CpG sites. *Nature* **401**, 301–304 (1999).
117. Riccio, A. *et al.* The DNA repair gene *MBD4* (*MED1*) is mutated in human carcinomas with microsatellite instability. *Nature Genet.* **23**, 266–268 (1999).
118. Prokhortchouk, A. *et al.* The p120 catenin partner Kaiso is a DNA methylation-dependent transcriptional repressor. *Genes Dev*. **15**, 1613–1618 (2001).
119. Wang, H. *et al.* Purification and functional characterization of a histone H3-lysine 4-specific methyltransferase. *Mol. Cell* **8**, 1207–1217 (2001).
120. Nishioka, K. *et al.* Set9, a novel histone H3 methyltransferase that facilitates transcription by precluding histone tail modifications required for heterochromatin formation. *Genes Dev*. **16**, 479–489 (2002).
121. Rea, S. *et al.* Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature* **406**, 593–599 (2000).
122. Yang, L. *et al.* Molecular cloning of ESET, a novel histone H3-specific methyltransferase that interacts with ERG transcription factor. *Oncogene* **21**, 148–152 (2002).
123. Schultz, D. C., Ayyanathan, K., Negorev, D., Maul, G. G. & Rauscher, F. J. III. SETDB1: a novel KAP-1-associated histone H3, lysine 9-specific methyltransferase that contributes to HP1-mediated silencing of euchromatic genes by KRAB zinc-finger proteins. *Genes Dev*. **16**, 919–932 (2002).
124. Ogawa, H., Ishiguro, K., Gaubatz, S., Livingston, D. M. & Nakatani, Y. A complex with chromatin modifiers that occupies E2F- and Myc-responsive genes in G0 cells. *Science* **296**, 1132–1136 (2002).
125. Peterson, C. L. Chromatin remodeling: nucleosomes bulging at the seams. *Curr. Biol.* **12**, R245–R247 (2002).
126. Reyes, J. C. *et al.* Altered control of cellular proliferation in the absence of mammalian brahma (SNF2α). *EMBO J.* **17**, 6979–6991 (1998).
127. Roberts, C. W., Galusha, S. A., McMenamin, M. E., Fletcher, C. D. & Orkin, S. H. Haploinsufficiency of Snf5 (integrase interactor 1) predisposes to malignant rhabdoid tumors in mice. *Proc. Natl Acad. Sci. USA* **97**, 13796–13800 (2000).
128. Kim, J. K. *et al. Srg3*, a mouse homolog of yeast *SWI3*, is essential for early embryogenesis and involved in brain development. *Mol. Cell. Biol.* **21**, 7787–7795 (2001).

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