Genetics and Epigenetics

REVIEW

Unexplored Potentials of Epigenetic Mechanisms of Plants and Animals—Theoretical Considerations

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Abstract: Morphological and functional changes of cells are important for adapting to environmental changes and associated with continuous regulation of gene expressions. Genes are regulated–in part–by epigenetic mechanisms resulting in alternating patterns of gene expressions throughout life. Epigenetic changes responding to the environmental and intercellular signals can turn on/off specific genes, but do not modify the DNA sequence. Most epigenetic mechanisms are evolutionary conserved in eukaryotic organisms, and several homologs of epigenetic factors are present in plants and animals. Moreover, in vitro studies suggest that the plant cytoplasm is able to induce a nuclear reassembly of the animal cell, whereas others suggest that the ooplasm is able to induce condensation of plant chromatin. Here, we provide an overview of the main epigenetic mechanisms regulating gene expression and discuss fundamental epigenetic mechanisms and factors functioning in both plants and animals. Finally, we hypothesize that animal genome can be reprogrammed by epigenetic factors from the plant protoplast.

Keywords: epigenetic mechanisms, environmental signals, DNA methylation, histone acetylation, gene expression, reprogramming, protoplast

Genetics & Epigenetics 2013:5 23–41

doi: 10.4137/GEG.S11752

This article is available from http://www.la-press.com.

Epigenetics—A Brief History

In recent years, a new research field has emerged known as epigenetics, which studies the factors that influence the function of genes. The term "epigenetics" was suggested by the developmental biologist Waddington in 1942, who used this phrase as a "study of the processes by which genotype gives rise to phenotype" without changes at the level of the gene itself.[1] However, the word "epigenetics" was used by Heinemann in the 19th century. The original concept can be traced back to Aristotle,[2] who proposed a new theory known as epigenesis, which means to grow upon genesis, the opposite of preformation. Today, according to the most accepted definition, epigenetics is the study of alterations in gene expression without changes in the DNA sequence, hence the name epi- (Greek: επί- over, above, outer) -genetics.[3]

One example of epigenetic changes in eukaryotic biology is the process of cellular differentiation by which a single totipotent egg cell develops into various pluripotent cell lines of the embryo, which in turn become fully differentiated cells. Epigenetics also examines the role of the environment in gene expression to determine how the environment can influence the expression of genes.[4]

This review, after describing some of the common epigenetic mechanisms of plant and animal cells, proposes potential epigenetic reprogramming mechanisms between plant and animal cells that have not been discussed in such reviews.

Epigenetic Regulation of the Genome

It has been well-established that DNA is organized by histones and non-histone proteins into chromatin.[5] Approximately 146 base pairs of DNA wraps around a complex structure of eight histone proteins (octamer) to form one bead on a chain of bead-like nucleosomes connected by 80-base pair linker-DNA. Histones are responsible for protection of DNA as well as maintaining the shape and structure of a nucleosome. There are five families of histones known to date, including H1/H5, H2A, H2B, H3, and H4.[6–8] H2A, H2B, H3, and H4 are the core histones, while linker DNA is associated histones H1 and H5. Nucleosomes act as a physical barrier to transcription factors that bind to certain regions of DNA. However, specific acetylation can remove the positive charge on the lysine amino group that is acetylated, so that the nucleosome becomes loosened on the DNA.[9]

It has long been hypothesized that the linker histones H1 and H5 are essential for chromatin condensation; however, this dogma has not been supported by studies. Knockout experiments in Tetrahymena and *Aspergillus nidulans* showed that H1 is not essential for nuclear assembly. Moreover, H1 was found to control gene expression through activation and repression mechanisms.[10,11] Each of the histones has an N-terminal tail with a specific sequence of amino acids, but the H2A also has a C-terminal tail.[12] The C-terminus forms a globular docking domain that is packaged into the core.[13] Several studies have demonstrated the importance of the histone tails for nucleosome remodeling by ATP-dependent chromatin remodeling factors.[14,15]

Histone N-termini undergo posttranslational modifications that alter their interaction with DNA and nuclear proteins. Such modifications include methylation,[16] acetylation,[17] phosphorylation,[18] sumoylation,[19] ubiquitination,[20] and ADP-ribosylation.[21] These modifications determine the interaction between the histone and other proteins, which may in turn regulate chromatin structure, and transcription.

Among core histones, the H2A family exhibits the highest sequence divergence, resulting in the largest known number of variants. These variants, found in nearly all organisms, include H2A.Z and H2A.X.[22] H2A.Z is associated with the promoters of actively transcribed genes and is also involved in the prevention of the spread of silent heterochromatin.[23] It has also been found that the chromatin remodeling complex SWR1 catalyzes ATP-dependent exchange of H2A in the nucleosome for H2A.Z.[24]

In the other hand, H2AX, another histone variant contributes to the detection, signaling and repairing of DNA double-strand breaks.[25] Plants exhibit a special class of H2A isoforms with an extended C-terminus comprising SPKK motifs.[26,27] Histone H3 and H4 are nearly identical in plants and animals. For instance, only two amino acids of the 102 amino acids of histone H4 differ between pea and calf thymus.[28] The linker histones are similarly found in all eukaryotes. The chromatin structure is therefore essential for both preventing of DNA and regulating gene expression, thereby preventing/enhancing

the binding of transcription factors, activators, and chromatin remodeling complexes to DNA.[29]

Fundamental Epigenetic Mechanisms

Epigenetic mechanisms are responsible for several phenomena, such as X-inactivation, genomic imprinting, and reprogramming.[30,31] There are several epigenetic processes, such as methylation, acetylation, and others that modify chromatin structure.[32] The main epigenetic processes are summarized in Figure 1. Generally, methylation is associated with heterochromatic gene silencing, while acetylation is associated with euchromatic gene activation.[33,34] A notable exception to general rule is methylation

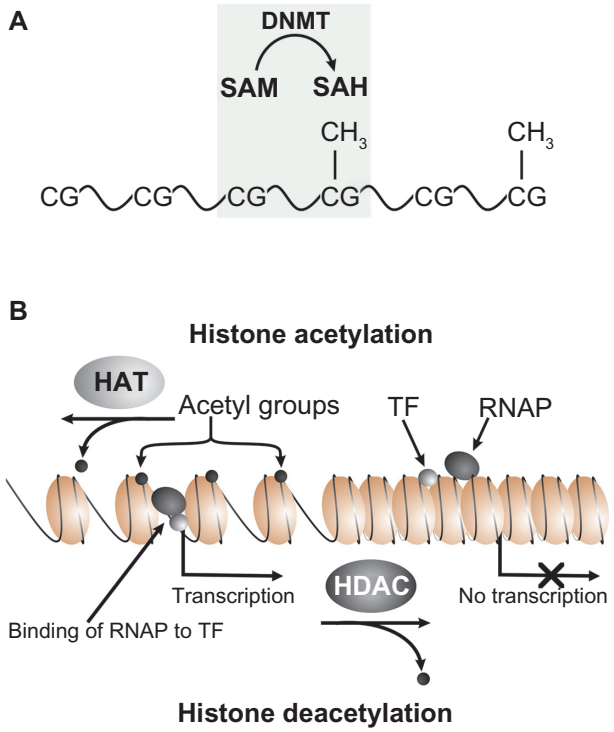

Figure 1. Basic epigenetic processes controlling gene expression. (**A**) DNA methylation, by which a methyl group (CH_3; light blue) is added to DNA nucleotide, occurs at CpG sites. The reaction is catalyzed by DNA methyltransferases (DNMTs) that transfer a methyl group from the S-adenosyl methionine converting cytosine to 5-methylcytosine. DNA methylation causes gene silencing (SAH—S-adenosylehomocysteine).(**B**) Histone modifications include acetylation—deacetylation, methylation—demethylation processes of histones. During acetylation, histones are acetylated (light green) on lysine residues in the N-terminal tail, thereby making DNA accessible for transcription. The opposing process is deacetylation of acetylated histones, making DNA inaccessible to RNA polymerase II (RNA pol II) and thus inhibiting transcription. Histone acetylation is catalyzed by histone acetyltransferases (HATs), while histone deacetylation is catalyzed by histone deacetylases (HDACs). During histone methylation, a methyl group is transferred to the histone tail by histone methyltransferases (HMTs), turning the genes "off", as in case of DNA methylation and histone deacetylation. Removal of methyl group from the histone tail catalyzed by histone demethylase (HDMs), turning genes "on". The figure was drawn based on references[33–37].

of some lysine and arginine residues of histones that leads to gene expression.[35,36] DNA and histone modifications by methylation/demethylation influence gene expression by making DNA inaccessible (by adding a methyl group to the DNA or histone tail) or accessible (by removing it) for transcription factors and other proteins. DNA methylation is implicated in fundamental processes such as genomic imprinting, X-chromosome inactivation, and in some diseases.[37] In fact, during ontogenesis, these processes regulate differentiation and determine which embryonic stem cell lines should differentiate from the totipotent zygote. The main epigenetic mechanisms regulating gene expression include the modification of DNA, the modification of histone proteins, and the chromatin remodeling.

DNA modification

DNA methylation is a crucial epigenetic modification of the genome that involves the addition of a methyl group to the N6 position of adenine or N4 or C5 position of cytosine.[38] DNA methylation is involved in regulating many cellular processes including embryonic development, chromatin structure, X-chromosome inactivation, genomic imprinting, and chromosome stability.[30,31,39] This mechanism is catalyzed by DNA methyltransferases (DNMTs) that transfer a methyl group to DNA by using S-adenosyl methionine (SAM) as the methyl donor. Operation of DNMTs leads to conversion of cytosine to 5-methyl cytosine, which suppresses gene expression. DNA methylation is performed by "de novo" methyltransferases that methylate previously unmethylated cytosines, while maintenance of methylation is performed by "maintenance methyltransferases". Maintenance of methylation refers to maintaining the methylation pattern once it is established.[40,41]

There are some diseases associated with aberrant DNA methylation, such as hyperhomocysteinemia, characterized by a high level of homocysteine in the blood, leading to vascular inflammation.[42] Metabolic disorders, such as diabetes (and obesity), have also been linked to aberrant DNA methylation;[43] moreover, it is involved in neurodegenerative disorders.[44]

Several data suggest that mechanisms of epigenetic regulation are general among eukaryotes, and even in prokaryotes. Enzymes that catalyze the mechanisms are highly conserved among eukaryotes.[45]

DNMTs catalyzing DNA methylation can be divided into three different groups, including those that generate N6-methyladenine (m6A), N4-methylcytosine (m4C), and C5-methylcytosine (m5C), each widely used by prokaryotes, fungi, plants, and animals.[46] The m6A and m4C DNMTs are found primarily in prokaryotes; however, the presence of m6A in certain fungi, algae, and several ciliates has been demonstrated.[47–49] The m5C DNMTs can be found in prokaryotes and eukaryotes, with 10 conserved motifs found in the catalytic domain of all DNMTs, suggesting a common origin.[50,51]

Based on sequence homology, DNMTs can be divided into at least 6 distinct classes, the DNMT1/methyltransferase 1 (MET1) class, DNMT2 class, DNMT3/domains rearranged methyltransferase (DRM) class, chromomethylases (CMT) class, MASC1/RID class, and MASC2/DIM2 class.[52] DNMT1 was first identified in animals; however, a homolog is also found in plants. In mammals, DNMT1 is thought to be a maintenance methyltransferase, while methylation in plants is maintained by a DNMT1 homolog methyltransferase MET1.[53–57] DNMT2 is the most conserved DNMT in eukaryotes[58] that contains all the conserved methyltransferase motifs and is involved in methylation of tRNA.[59] DNMT2 was found to have the same function in mammals and flowering plants.[60] Interestingly, DNMT2 is also involved in histone deacetylation in Arabidopsis thaliana, a favorite model for plant biologists, suggesting that it participates in epigenetic regulation in plants.[61] Nevertheless, very little is known regarding the function of DNMT2 in epigenetic regulation in plants and in animals. Recognition of hemimethylated DNA is catalyzed by variant in methylation (VIM) proteins in plants and ubiquitin-like, with PHD and RING finger domains 1 (UHRF1) proteins in animals.[55–57]

Unlike mammalian DNMT1, members of the DNMT3 subfamily (eg, DNMT3a and DNMT3b) as de novo methyltransferases are responsible for establishing cytosine methylation patterns at unmethylated DNA.[62] In plants, DNA methylation is established by DRM2, which is a DNMT3 homolog.[45]

Demethylation of the DNA can take place through passive and active processes. Passive DNA demethylation occurs when cells fail to maintain their methylation state during DNA replication. Active DNA demethylation is primarily established by a small group of glycosylases, eg, repressor of silencing1 (ROS1), Demeter (DME) and Demeter-like3 (DML3) in plants, and 5-methylcytosine hydroxylases in animals, which introduce an abasic site.[63,64]

Epigenetic factors involved in DNA modification found in plants and animals are summarized in Table 1. It was reported in Arabidopsis, a regulator of DNA demethylation, IDM1, is required for preventing DNA hypermethylation, whereby binding methylated DNA at chromatin sites lacking histone methylation and acetylation protects genes from silencing.[65] In animals, active DNA demethylation occurs after a sperm enters an egg.[66] It was recently reported that expression of unmethylated plasmids was detected in a mouse embryo $<$12 h after in vitro methylated plasmid injected into the zygote. The expression of methylated plasmids was delayed until the 8 cell-stage.[67] This suggests that DNA demethylation plays a critical role in regulating development both in plants and animals.

Histone modification

Acetylation is catalyzed by histone acetyltransferases (HATs). Histone acetylation enhances transcription by converting the positively charged lysine residues in the N-terminal tail into neutral residues, resulting in the loosening of the bond between DNA and the histone (Fig. 1). HATs acetylate N-terminal lysines on histones H2B and H3. Nuclear HATs are classified into several families, including the GCN5 (general control non-repressed protein5)-related N-acetyltransferase (GNAT) family. A study found that one member of the three subfamilies of GNAT is present in plants, animals, and fungi, suggesting functional conservation.

Histone deacetylases (HDACs) remove acetyl groups from an N-acetyl lysine amino acid on a histone. In plants, histone deacetylase HDA2 is a homolog of the animal HDAC11. An HDAC class, designated as class 3, has been identified within the reduced potassium dependency3/histone deacetylase 1 (RPD3/HDA1) family found only in plants and animals by phylogenetic analysis.[68]

Chromatin remodeling

Chromatin remodeling implies the assembly and disassembly of the nucleosomes, ATP-dependent chromatin remodeling, and modifications of histones.[69] Nucleosome assembly factors, such as chromatin

Table 1. Summary of DNA modifications in plants and animals.

Epigenetic mechanism	Factor		Function/comment	Reference
	Plant	Animal		
Maintaining methylation	MET1 VIM	DNMT1 UHRF family	Transfer of a methyl group to DNA Establishment of DNA methylation patterns, recruitment of the maintenance Dnmt1/Met1 to hemimethylated DNA	55 209 56,57,210
De novo methylation	DRM2	DNMT3	Drm2 maintains CHH or asymmetrical methylation through a small interfering RNA (siRNA)-driven signal in a process known as RNA-directed DNA methylation	41,211
Demethylation	DME (ROS1, DML2,3)	DME Gadd45a	Dme members have not yet been identified in animals	210,212

Notes: In plants, DNA methylation commonly occurs at cytosine bases within all sequence contexts. CHH—Asymmetric CHH context, where H = A, T, or C.
Abbreviations: Met1, Maintenance DNA methyltransferase1; DNMT1, DNA methyltransferase1; VIM, Variant in methylation; UHRF, Ubiquitin-like PHD and RING finger domain; DRM2, Domains rearranged methyltransferase2; DNMT3, DNA methyltransferase3; DME, Demeter; ROS1, Repressor of silencing1; DML2,3, Demeter-like2,3; Gadd45a, growth arrest and DNA damage-inducible protein 45.

assembly factor1 (CAF1) and nucleosome assembly protein1 (NAP1), facilitate transcriptional regulation through deposition of histones H3 and H4 onto DNA both in plants and animals.[70] Chromatin-remodeling-enzymes are ATP-dependent and responsible for conformational changes in chromatin.[71,72] The subunits of chromatin-remodeling-complexes contain different domains (bromodomains, chromodomains, PHD fingers) that are involved in transcription. The members of the ATPase-dependent chromatin-remodeling-enzymes, for example the switch/sucrose nonfermentable2 (SWI2/SNF2), the imitation switch (ISWI), and the chromodomain-helicase-DNA-binding protein (CHD) groups, have ATPase domains that are responsible for their chromatin remodeling activity.[73] Chromatin-remodeling-enzymes use nucleosomes as substrates and change positions of histone octamers and/or the topology of DNA that is wrapped around the nucleosome particles. The SWI/SNF family of chromatin-remodeling ATPases is conserved among the plants and animals; moreover, the SNF2 subfamily of the SWI/SNF complex is found in organisms from yeast to human.[74] A summary of various epigenetic factors involved in histone modification and chromatin remodeling processes found in animal and plant cells are provided in Tables 2 and 3.

Homolog factors in plants and animals regulating gene expression

The order of the organs and body parts are determined genetically. Homeosis is the transformation of one body part into another arising from mutation of specific developmentally critical genes.[75] Homeosis were first recognized in plants (Linnaeus, Goethe) and genetic studies on homeotic mutants of plants were carried out on Arabidopsis and Antirrhinum in the late 20th century.[76] However, breakthrough discoveries were made in animals. Homeotic genes are conserved master genes that switch on early during ontogenesis and remain in a stable active or inactive state throughout life. These genes are involved in developmental patterns and sequences; for example, they are involved in determining when, where, and how body segments develop in flies.[77] The proteins encoded by the Polycomb group (Pc-G) form structures similar to the heterochromatin and inactivate specific genes such as Hox genes.

In animals, Pc-G and trithorax group (Tr-G) proteins are responsible for continuous activity/inactivity of homeotic genes: members of the Tr-G help to fix homeotic genes into the active state, while Pc-G proteins are in the inactive state.[78] Some studies reported functional similarities between plant and animal Polycomb complexes. The Polycomb repressive complex 2 (PRC2) proteins enhancer of zeste [E(z)], extra sex combs (ESC), and suppressor of variegation [Su(z)12] of mammals are involved in repression of homeotic genes. In plants, proteins such as fertilization-independent endosperm (FIE), curly leaf swinger (CLF/SWN), multi-copy suppressor of ira (MSI), and embryonic flower2 (EMF2) form PRC2-like complexes have a very similar function: to maintain the repressive condition of plant homeotic genes.[69,79] A PRC2 complex carries out trimethylation

Table 2. Summary of histone modifications in plants and animals.

Epigenetic mechanism	Factor		Function/comment	Reference
	Plant	Animal		
Acetylation	HATs (eg, GNAT, p300/CBP)	HATs (eg, GNAT/MYST, HAC1)	HATs not only specify histone modification, but also transcriptional function	213–218
Deacetylation	HDAC1 (RPD3/HDA1)	HDAC1 (RPD3/HDA1)	RPD3/HDA1-like HDACs are found in all eukaryotic genomes	68,219–221
Methylation	HMTs (SET domain proteins: CLF/SDG1, SWN/SDG1, MEA/SDG5)	HMTs (SET1, ASH1)	SET domain found from yeast to human	222,223
Demethylation	JMJC proteins (eg, KDM7B)	JMJC proteins (eg, JHDM1)	JHDM1 demethylates histone H3 at lysine 36; PKDM7B demethylates trimethyl H3K4	224,225

Abbreviations: HATs, histone acetyltransferases; GNAT, Gcn5-related N-acetyltransferase; p300/CREB, p300/CREB-binding protein; MYST, Named for the founding members MOZ (MYST3; MIM 601408), yeast YBF2 and SAS2, and TIP60 (HTATIP; MIM 601409); HAC1, Histone acetyltransferase1; HDAC1, Histone deacetylase1; RPD3, Reduced potassium dependency 3; HDA1, Histone deacetylase 1; HMTs, Histone methyltransferases; SET, [Su(var)3-9, E(z), Trx]; CLF/SDG1, Curly leaf/set domain group1; SWN/SDG1, Swinger/Set domain group1; MEA/SDG5, Medea/Set domain group5; SET1, [Su(var)3-9, E(z), Trx]1; ASH1, Discs absent, small, or homeotic-1; JMJC proteins, Jumonji domain-containing proteins; KDM7B, histone lysine (K) demethylase7B; JHDM1, JmjC domain-containing histone demethylase 1.

Table 3. Summary of chromatin remodeling in plants and animals.

Epigenetic mechanism	Factor		Function/comment	Reference
	Plant	Animal		
Chromatin remodeling	CAF1 (eg, FAS1,2), NAP1, MSI1, HIRA	CAF1 (eg, p150, p60, p48), HIRA	Chromatin assembly, disassembly, in animals, HIRA functions as a chaperone of the variant histone H3.3	69,74,226,227
	ISWI	ISWI (SNF2H)	Facilitate sliding of histone octamers on the DNA	228
	SWI/SNF (eg, BRM, SYD), CHD (eg, PKL)	SWI/SNF (eg, BRM, BRG1, SWI2/SNF2, CDH (eg, CHD1-4,9, NURD)	SWI/SNF complexes facilitate deacetylation of histones CHDs are both transcriptional repressors and activators	229–232 233

Abbreviations: CAF1, chromatin assembly factor1; FAS1,2, fasciata1,2; NAP1, nucleosome Assembly Protein1; MSI1, multicopy suppressor of ira1; HIRA, histone repression a factor; ISWI, imitation switch; SNF2H/L, sucrose nonfermenting 2 homolog; SWI/SNF, switch/sucrose nonfermentable; BRM, brahma; SYD, splayed; CHD, chromodomain-helicase-DNA-binding protein; PKL, pickle; BRG1, brahma-related gene1; SWI2/SNF2, switch2/sucrose nonfermentable2; CHD1-4,9, chromodomain-helicase-DNA-binding protein1-4,9; NURD, nucleosome-remodeling and histone deacetylation.

of H3Lys27, which correlates with the heterochromatic gene silencing in humans and possesses the evolutionary conserved SET domain.[80,81]

The Su(var)3-9, E(z), Trx (SET) domain was first identified as a conserved sequence of three Drosophila proteins (Su(z), E(z), trithorax) and is highly conserved in all eukaryotes.[82] The TrxG protein of SET domain catalyzes methylation of H3K4, which plays a role in transcription activation in both animals and plants.[83–85]

The members of the SWI/SNF family, such as ATP-dependent chromatin remodeling complexes (CRCs), are highly conserved in both animals and plants.[86] The ATP-dependent CRCs function depends on their ATPase and play crucial roles in regulating transcription (activation, repression), differentiation, and ontogenesis by controlling the accessibility of DNA sequences to transcription factors.[87] There is a great similarity among SWI/SNF subunits, which are homologous with those in Saccharomyces cerevisiae SWI3 in mammals and plants.[88] It has been demonstrated that mammalian SWI/SNF like BRG1-associated factors (BAFs) play a crucial role in the formation of embryonic toti- and pluripotent stem cells. Embryonic stem cells express a factor distinguished as esBAF, which is defined by the presence of human BAF155 genes. Mice homozygous to the null mutation of BAF155 die during the pre-implantation stage, and heterozygous mutants develop with neural tube defects.[89] Mutation of an Arabidopsis homolog of BAF155, AtSWI3 leads to inhibited development at the globular stage.[90,91]

The Jumonji transcription factor (Jmj) family was first identified in mouse whose members are involved in histone modification.[92] It was reported that the JmjC domain containing transcription factors demethylate histones in both animals and plants. However, it was also shown that plant JmjC has both conserved and specific functions, in contrast to in mammals.[93–95]

Specific markers, such as sex-determining region Y-box 2 (Sox2), octamer-binding transcription factor 4 (Oct4) and Nanog, can characterize pluripotent embryonic stem cells. As in animal cells, the plant meristematic cell state is maintained by transcription factors, including WUSCHEL (Wus), CLAVATA (Clv), and PLETHORA (Plt). A HAT complex GCN5 is essential for maintaining of root stem cell niche through the attenuation of Plt.[96] In animals, the proto-oncogene c-Myc has a very similar function. In addition, it regulates the expression of GCN5 via binding to the GCN5 promoter.[97]

Epigenetic Mechanisms Regulating Nuclear Reprogramming—Significance of Plasticity

Epigenetics is strongly related to nuclear reprogramming; in fact, it can be considered as the base of genetic reprogramming. The term nuclear reprogramming is used to describe changes in gene activity that are induced naturally (fertilization) or experimentally by introducing nuclei into a new cytoplasmic environment.[98] Experimentally induced nuclear reprogramming has a close link to cloning. The term clone (ancient Greek: κλώνος [klonos], meaning "twig") had already been used since the beginning of the 20th century in reference to plants, and later to animals.[99] This can be done by removing cells from the roots of the plant and place them in a solution containing nutrients; thus, a large number of undifferentiated cells can be generated, known as the callus.[100] Cells of the callus are totipotent, meaning that they have the ability to develop into any other cell type.[101] Addition of plant hormones, such as auxins, cytokinins, and gibberellins to these cells results in the development of a whole plant that is genetically identical to the original plant.[102]

Cloning of animals is a much more complex problem because differentiated animal cells are less susceptible to dedifferentiation. However, cloning is not only observed in plants, but also several animals (aphides, fishes, lizards, frogs, etc) can reproduce without fertilization under natural conditions, cloning themselves as a part of their natural life cycle. This means that even in animals, a type of "natural cloning" is possible, which is produced by mitosis of the germ line cells of the (female) parent.[102–106]

The theory, originating from the great theoretician August Weismann (1834–1914) that cells forming tissues lose their reproductive ability and death becomes the natural part of their life cycle, persisted for a long time. The origin of this theory goes back to the end of the 19th century, when Weismann proposed the so called "germ plasma" (Keimplasma) theory, stating that germ line cells develop separately from somatic cells, thus indicating that acquired characteristics

cannot be inherited. In many animals, there are special granules containing ooplasmic determinants in the egg which are responsible for the determination of the germ line cells.

The German embryologist Wilhelm Roux (1850–1924) stated that when the first cleavage division separated the future right half of the embryo from the future left half, there would be a separation of "right" determinants from "left" determinants in the resulting blastomeres. To test the hypothesis, in 1888 Roux used two-cell frog embryos and killed one of the cells of each embryo with a hot needle. Thus, he obtained half-embryos. Based on these and theoretical deductions, the two great scientists created a long lasting, but incorrect hypothesis: according to the Roux-Weismann theory, the diversity of the cell fates is due to the segregation of nuclear determinants during cleavage divisions, so cell nuclei become different both quantitatively and qualitatively, ascribed to the loss of genetic material.

The development of nuclear transfer experiments overthrew this thesis. In the 1930s, it was examined whether genomes of terminally differentiated cells could be reprogrammed. To answer this question, the Nobel Prize Laureate German embryologist Hans Spemann proposed an experiment: differentiated cell nuclei should be transplanted into enucleated egg cells (considered science fiction at the time of the proposal). If each cell nucleus is genetically identical to the zygote, the transplanted nucleus should be able to initiate, drive, and control the development of a new organism.[107] Unfortunately, until the early 1950s, the technical background was not available to carry out these experiments and test this idea. However, in 1952, Briggs and King successfully cloned 27 tadpoles from 104 nuclear transfers in northern leopard frogs.[108] In 1958, John Gurdon transferred intact nuclei from somatic cells into a Xenopus oocyte and successfully cloned a frog. He stated that the nucleus of a fully differentiated cell can return to a pluripotent state.[109] In the 1960s, he also produced frogs from gut epithelial cells, yet some scientists remained skeptical, debating the fact that gut cells were differentiated and suggested that they were primordial germ cells seated in the epithelium.[107] Until now, these significant experiments were praised and attributed significantly to his earning of the Noble prize in 2012, whereby many independent studies showed that fully differentiated cells can regain their totipotency.

Gurdon's experiments were the first demonstration in animals that the nucleus of a differentiated somatic cell can regain the potential to differentiate into any cell type. Additionally, regeneration experiments showed that mature cells can be transformed into other mature cells without going through an intermediate pluripotent state.[110–113] The conversion of one cell to another is termed metaplasia and includes either conversions between stem cells or direct conversion of differentiated cells, respectively.[114–116] Transdifferentiation (also known as lineage reprogramming) is a type of metaplasia defined as irreversible conversion of already differentiated cell to another cell type, resulting in the loss of one phenotype and the gain of another.[117] For transdifferentiation, lens regeneration, also known as Wolffian regeneration (Wolff, 1895), was demonstrated as well as metaplasia liver-to-pancreas metaplasia.[118] Also in the liver, for a previous study demonstrated transdifferentiation by converting hepatocytes into bile duct cells.[119] It has recently been found that using ectopic transcription factors, adult dermal fibroblasts can be converted into neural progenitor cell-like cells (iNPCs) with similar properties as primary NPCs.[120]

In 1996, the doctrine that the genetic material of fully differentiated cells is no longer able to produce an adult organism was finally disproved, when Scottish scientists, including the biologist Ian Wilmut, cloned a lamb successfully at the Roslin Institute using adult mammary epithelial cells incubated in depleted serum to "synchronize" to the mitotically "slow" oocyte, from a mature ewe as nucleus donor, and was transplanted to an enucleated oocyte.[121] The cloning was easily verified because the phenotype and genotype of the cloned offspring could be clearly distinguished from the foster mother's characters. The difficulty in these procedures was cleared by the fact that only 29 from the 270 nuclear transfers resulted in embryos, and just one survived. However, the only surviving sheep, Dolly, was able to produce healthy offspring, including Bonnie. Later, an entire series of mammals were successfully cloned.[122]

However, there are many negative aspects of cloning, including low rates and regulation problems. Cloning is very inefficient since most clones die soon after implantation.[123,124] Even clones that survive

often have serious abnormalities (eg, increase of body weight, kidney hypertrophy), mutations, and shortened life spans, likely a corollary of the age of somatic cells used for cloning.[123,125–127]

An important question arises: can a stem cell be created from a somatic cell without the need for human eggs? Two Japanese researchers, Takahashi and Yamanaka, demonstrated that ESC-like cells could be induced by some transcription factors, including Sox2, Oct4, Kruppel-like factor 4 (Klf4), and c-mycooncogene (c-Myc) pluripotency factors (also known as Yamanaka factors).[128] Nuclear reprogramming using transcription factors may resolve the ethical problems related to embryonic stem cells. This method would enable development of pluripotent stem cell-based regenerative medicine. However, inducing of iPSC is very complicated and the risk of obtaining undesired cells indicates that further studies should be conducted before this method can be widely applied.[129]

The importance of the discovery by Gurdon[130] that specialization of cells is reversible and by Shinya Yamanaka[128] that intact mature cells can be reprogrammed to become immature stem cells is acclaimed by award of Nobel Prize in Physiology or Medicine in 2012. Notably, there is a great difference between the cellular plasticity of plants and animals. Cellular plasticity is the ability of cells to change their structure or function to become a different type of cell is, as we understand today, depending on the epigenetic regulation of gene expression (Fig. 2). Plasticity of plant cells to transdifferentiate into various types of cells is much higher than that of animal cells, which implies a much "looser" chromatin structure. This, however, should not be interpreted that the chromatin structure is less complex or that it is more complicated to regulate.[131,132] Further in vitro epigenetic experiments and in vivo experiments, such as xenotransplantation, may reveal this phenomenon. Based on experimental results, it may be possible to reprogram a fully differentiated animal cell nucleus using a recipient plant protoplast, a hypothesis which should be verified by future research.

Conserved Epigenetic Mechanisms in Cells of Plants and Animals

The functional parallels between epigenetic elements of plant and animal development suggest that a high

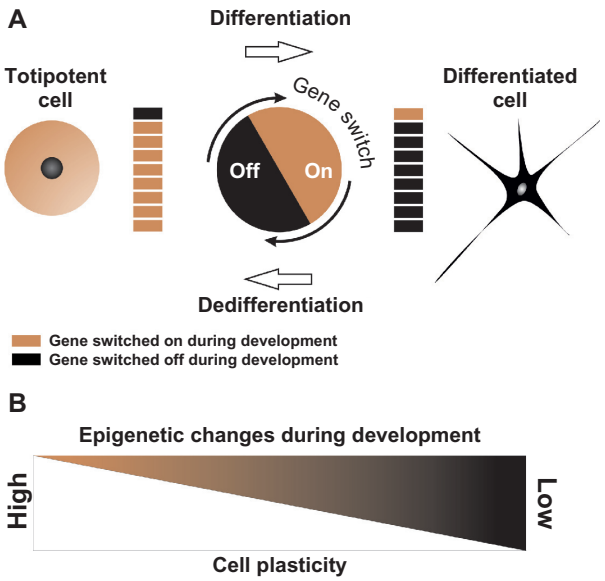

Figure 2. Changes in cellular plasticity. (**A**) Gene silencing and activation during differentiation and dedifferentiation. In a totipotent cell, such as the fertilized egg, genes responsible for segmentation and formation of pluripotent embryonic cells are switched on. Throughout differentiation, early genes are switched off, while genes needed for differentiated cell functions are switched on and others are switched off or repressed. Repressed genes can be activated reprogramming somatic cells, eg, neuron to totipotent or pluripotent states. (**B**) Epigenetic modifications or cell plasticity enables stem cells to differentiate into various cell types or differentiated cells to trans-differentiate to each other. During differentiation, cell plasticity is decreased. Differentiated cells have low plasticity; however, high plasticity can be increased by adding extrinsic factors that affect epigenetic processes, even in completely differentiated cells.

congruence in the epigenetic mechanisms is present in plants and animals. It should be mentioned, however, that although there is a high conservation of homeotic genes, the role of homeotic genes is dramatically different in the two groups.

In animals, the expression pattern of some 'classical' homeotic genes forming gene clusters is the basis for the concept "zootype", which means that all animal phyla shared a particular pattern of gene expression. In plants, these genes are not homologous to "classical" Hox genes, suggesting that the functions of homeotic genes developed independently in the evolution of plants and animals.[133,134] Comparing epigenetic patterns at the molecular level of plants and animals shows that they possess similar patterns, which are more pronounced at molecular level than at the phenomenological level. Both taxons use the same processes for epigenetic regulation, and sometimes surprising similarities are present with respect to epigenetic factors. Several examples of proteomic analysis show that a homologous transcription factor present in one group can substitute for another that

is absent.[93–95] Some experiments have also demonstrated that the two distant epigenetic systems may be congruent to some extent.[135,136]

Although several data suggest that the epigenetic regulation among plants and animals appears to be similar, an increasing amount of recent data has shown that there are many differences between the two groups, necessitating further studies.[131,137]

RNA Epigenetics

It has been revealed that up to 90% of eukaryotic genome is transcribed, but only 1%–2% of these transcripts encode for proteins, while the vast majority are transcribed as non-coding RNAs (ncRNAs). ncRNAs such as micro RNAs (miRNAs) are evolutionarily conserved, approximately 21 nucleotides in length, and play crucial role in development, stress responses, and chromatin states. RNA epigenetics also shows some similarities between animals and plants. In animals, such as humans, miRNAs are synthesized as single-stranded RNAs and cleaved by the RNaseIII enzymes Drosha and Dicer, producing precursor microRNAs (pre-miRNAs) and finally miRNA/miRNA duplexes.[138] In plants, Dicer-like1 (DCL1) enzymes carry out these processes.[139] In both plants and animals, miRNAs post-transcriptionally regulate gene expression via interactions with their target mRNAs. A major difference between plant and animal microRNA is observed for target recognition. Animal miRNAs repress gene expression by mediating translational attenuation, while nearly all plant miRNAs regulate their targets by directing mRNA cleavage at single sites in the coding regions.[140] It has been demonstrated that miRNAs can also cause histone modification[141] and direct DNA methylation.[142,143] Interestingly, a recent study revealed that miRNAs of digested plants are present in the serum of healthy human.[144]

To support the cross-kingdom similarity of miRNAs with regard to epigenetic regulation of the genome, Vaucheret and Chupeau demonstrated in a recently study that ingested plant small RNAs directly influence gene expression in animals.[145]

Genomic imprinting

Genomic imprinting is an epigenetic process by which certain genes are expressed in a sex-dependent manner.[146] It includes DNA and histone modification processes.[147,148] Genomic imprinting has independently evolved in flowering plants and mammals;[148,149] however, both in plants and animals, imprinting occurs in embryo-nourishing tissues, such as the placenta and the endosperm. Imprinted gene expression results from the sex-specific methylation of imprinted control regions (ICRs), such as differentially methylated regions (DMRs) in the parental germlines both in plants and mammals.[150,151] Imprinting is carried out by DNA methylation and Polycomb group-mediated trimethylation of histone H3 at lysine 27 (H3K27me3) in mammals[152,153] as well as in plants.[154–156] However, control of imprinting differs between plants and animals.[157]

Role of Microenvironment in Cell Fate, Differentiation, and Dedifferentiation

In addition to epigenetic factors inside the cell, the fate of cell lineage and differentiation require continuous communications between the microenvironment of the cell, ie, extrinsic factors, extracellular matrix, and signals from neighboring cells, and the cell itself.[158–160] Interactions between cells, physical conditions, and mechanical forces are also important for cell fate decision and differentiation. An interesting experiment modeled the surface geometric pattern, which affects the development of stem cells. According to this study, the shape of cells increases compressive forces in the cytoskeleton with the result that most of the flower-shaped cells form fat tissue, while star-shaped cells form bone tissue.[161] In a previous experiment, the human ear was successfully grown on the back of a mouse using bovine chondrocytes with a human ear-shaped degradable polymer as a scaffold, which served as a geometric signal.[162] Changes in the environment may also affect differentiated cells. When a differentiated cell nucleus is transferred into a previously activated enucleated egg cell, genetic reprogramming is taking place. It is important to note that reprogramming is not one-sided, since when a cell nucleus is located in an atypical environment, the prevailing conditions exert inductive signals. This occurs when fully differentiated plant cells undergo cell wall degradation generating protoplasts which are totipotent.[163] In vitro experiments suggest that cell shape can influence cell fate determination of mesenchymal stem

cells between chondrogenic and smooth muscle cell lineages through cell adhesion molecules-mediated pathways.[164] Thus, extracellular stimuli, adhesion, and cell shape properties are critical determinants of cell fate and differentiation.

Epigenetic states of the cell can be modified by nutrition, behavior, stress, physical activity, and infections.[165–167] It has been established that early stress effects can elicit changes in adult gene expression through epigenetic processes. Additionally, maternal stress can determine the gene expression pattern in the adult. Weaver et al found that increased pup licking and grooming (LG) and arched-back nursing (ABN) by rat mothers altered the offspring epigenome at the glucocorticoid receptor (GR) gene promoter in the hippocampus.[168]

In addition, a plethora of examples in plants demonstrates the importance of changes in gene expression through epigenetic regulation in response to stress adaptation.[169–171] Additionally, a convincing example of adaptation to temperature stress by epigenetic regulation is the vernalization in plants growing at high altitudes.[172] It has been revealed that activity of flowering locus (FLC) gene having a principal role in vernalization response state is controlled by DNA methylation, which allows the mitotically stable inheritance of the vernalized plant.[173,174]

In animals, dietary supplements such as vitamins can also influence epigenetic processes by affecting enzymes that regulate methylation[175] or by regulating methyl-group transfer.[176] These effects influence the development of diseases, such as obesity.[177,178] Lack of folic acid has been shown to be associated with genomic hypomethylation[179] and neural tube defects.[180] Physical exercise can also influence epigenetic mechanisms, as reviewed by number of papers.[181,182] It has been revealed that physical activity may affect epigenetic regulation of tumor suppressor genes contributing to cancer survival.[183,184] Environmental exposure-induced abnormal epigenetic processes have been observed in many types of human[185–187] and plant[167] tumors. It is also important to note that miRNAs acting as tumor suppressor genes are involved in various stages of carcinogenesis.[188,189] Epigenetic upregulation of suppressor miRNAs, nutritional factors, particularly vitamins, such as vitamin A,[190,191] vitamin D,[192] vitamin E,[193] and folate[194] have been shown to prevent carcinogenesis.

Interplay between Epigenetic Mechanisms of Plants and Animals—In Vitro Chromosome Condensation and Nuclear/Nucleosome Assembly

Successful xeno-transplantation or xeno-cloning, the transplantation of cells, tissues, or organs from one species to another, has been well-documented in replication and division. For instance, DNA transcription and division was observed in human nuclei, when they were injected into amphibian oocytes.[195,196] The same phenomenon has not been documented between plants and animals. However, nuclear and chromatin assembly studies may deepen our understanding how to successfully conduct xeno-cloning between plant and animal.

As we described above, many transcription factors connected to the regulation of genes by rendering the chromatin state as active or silent are largely conserved in plants and animals. The ability of these transcription factors to access their binding sites depends on the structure of cellular chromatin.[132,197,198] Cell shape can influence cell fate determination; therefore, cellular chromatin can be changed by the cell's microenvironment.

In a previous study, nuclei from carrot were injected into maturing Xenopus oocyte as a recipient.[199] In the control experiment, an immature oocyte was used. Prematurely condensed nuclei with premature chromosome condensation was observed after introduction of either Xenopus brain nuclei or carrot protoplast nuclei into an in vitro matured X. laevis egg immediately after germinal vesicle break down (GVBD). No chromosome condensation was observed after introduction of either Xenopus brain or carrot protoplast nuclei into Xenopus oocytes prior to GVBD.[199] These findings suggest that chromosome condensation is restricted to mature oocytes that have undergone GVBD and that transplanted plant nuclei into Xenopus oocyte continues RNA synthesis, but this phenomenon was not observed when Xenopus somatic nuclei were injected into Xenopus oocytes. Breakdown of the nuclear membrane for both the plant and Xenopus nucleus by Xenopus cytoplast

factors was also detected,[199] suggesting that there is conservation between plants and animals regarding the enzymes involved in nuclear breakdown. However, it is possible the some nuclear damage also occurred during these procedures.

Plant Cytoplasm and Animal Chromatin

In vitro experimental evidence suggests that the plant cytoplasm is able to induce nuclear reassembly of the animal cell. For example, it was reported that carrot cytosol extract reassembled nuclear structure around a demembranated sperm chromatin from X. laevis.[200] In this experiment, demembranated sperm cells and membrane vesicle purified from X. laevis was introduced into plant cell cytosol extract from carrot, which supplied an ATP-regenerating system. Immediately after introduction, the demembranated sperm chromatin was in a long, thin, and highly condensed form and strongly stained with 4′,6-diamidino-2-phenylindole (DAPI). Incubation at a specific temperature demembranated by lysolecithin sperm exhibited a series of structural and morphological changes including elongation, swelling, decondensation, changing to a round shape, and a nucleus-like structure, finally acquiring a continuous double-layered nuclear envelop with nuclear pores.[200] After a long incubation, the newly assembled nuclei showed characteristics typical of normal nuclei. In the control, which contained DNase buffer, this phenomenon was not observed. Additionally, remodeling of the demembranated sperm chromatin based on the appearance of a typical DNA ladder after electrophoresis. Positive control freezing and thawing mouse liver nuclei showed a typical DNA ladder; as a negative control, lane 1 was loaded with sperm chromatin in DNase buffer. Using micrococcal nuclease did not result in DNA ladder in sperm, but a typical DNA ladder appeared in the carrot cell extract after micrococcal nuclease treatment, indicating that remodeling had occurred in this cell-free system.[200] However, whether advanced structures developed, such as solenoids, remains unresolved.[200]

Similarly, using a cell-free system purified from Nicotiana tabaccum ovules and demembranated X. laevis sperm, chromatin decondensation and nuclear membrane assembly were observed.[201] Demembranation was obtained in the same manner as in the study described above. In both cases, micrococcal nuclease digestion was used to verify nucleosome formation. Because Xenopus sperm is deficient in H1 histones, exposure to micrococcal nuclease leads to heterogeneous distribution of DNA fragment sizes.[201] When Xenopus sperm nuclei were incubated with Nicotiana ovule extracts, the chromatin proteins could be replaced by histones derived from Nicotiana ovules, resulting in remodeling of the chromatin structure.[201]

In both cases, nuclear remodeling and nucleosome assembly were observed, suggesting that transcription factors and/or cyclin-cdk complexes originating from the plant cytoplasm may contribute to the induction of nuclear reconstitution and chromatin formation. However, complex chromatin structures, such as solenoids, were not observed and no mitosis was detected.[200,201]

Animal Cytoplasm and Plant Chromatin

A similar condition was applied when genetic reprogramming was carried out between an algae and an amphibian.[135] In this experiment, chromosomes from the algae Crythecodinium cohnii were incubated in cytoplasmic extracts of unfertilized X. laevis oocytes or C. cohnii cell extracts. Introduction in cell-free extract from X. laevis resulted in chromosome decondensation and recondensation, nuclear membrane formation, and nuclear reconstitution. The newly assembled nuclei were morphologically different from the normal algae nuclei. Electron micrographs showed that the nuclear envelope of C. cohnii was discontinuous. However, the reconstituted nuclei possessed a normal membrane with nuclear pores which was morphologically indistinguishable from that of normal higher eukaryotic interphase nuclei. In contrast to the highly condensed chromosomes attached to the dinoflagellate C. cohnii nuclear envelope, the chromatin in the newly assembled nuclei dispersed uniformly, similar to that of typical higher eukaryotic interphase nuclei.[135] In addition, there was no nuclear assembly detected when C. cohnii chromosomes were introduced into cell-free extract from C. cohnii.

These experiments clearly showed that plants and animals can influence each other through their cytoplasm and show that induction of purified DNA/chromosomes with cell-free extract from other species can lead to nuclear and nucleosome/chromatin assembly.[135,200,201] However, these results do not preclude the mechanical/chemical microenvironmental effects on chromatin caused by the enucleation and

nuclear transfer. In addition, each described only nuclear and nucleosome assembly as a result of purified chromosome induction with cell-free extracts, which is not extraordinary. Furthermore, in vitro nuclear assembly is independent of nucleosome/chromatin assembly.[202] Early experiments demonstrated that cell-free extracts derived from species belonging to an amphibian class could induce formation of a nuclear envelope, chromatin decondensation, initiation of DNA synthesis, and chromosome condensation in sperm nuclei of X. laevis without membranes.[203] The experiments described here only revealed changes in the morphology of chromatins, but not changes in DNA synthesis and mitosis.

Unicellular algae dinoflagellata C. cohnii lacks histones, which may explain why nuclear assembly did not occur when purified chromosomes from C. cohnii were introduced into cell-free extract from C. cohnii.[135] In dinoflagellata, only three proteins possess similar biochemical traits as H4 in higher eukaryotes.[204] Other experiments demonstrated that cytoplasm and purified chromosomes isolated from plant and from animal can induce chromatin assembly via cytoplast factors involved in histone protein synthesis.[200,201] The Xenopus egg extract possesses two histone variants, histone H2A. X and histone B4, which correspond to H2A and H1 histones found in eukaryotic somatic cells.[205] In the experiments, the Xenopus egg was arrested in metaphase, indicating that full components or factors are necessary for chromatin decondensation and recondensation during nuclear assembly. However, the reasons for DNA replication failure remain unclear. However, it has already been reported that in animal cells, factors involved in chromosome condensation are associated with mitosis and meiosis.[206]

A Novel Hypothesis

Based on the studies described above, we can hypothesize that the donor nucleus from an animal cell can reprogram the cell fate and develop into a special animal cell—"green cell"—through epigenetic mechanisms and factors of the plant protoplast. Furthermore, it can be hypothesized that external stresses, such as cell wall/membrane removal and enucleation, elicit protoplast induction/activation, resulting in the release of nuclear transcriptional regulators, thereby influencing chromatin states of the transferred nucleus. In Figure 3, a hypothetical experiment is described, in which one can transfer an animal nucleus to an enucleated plant cell, ie, protoplast, to reprogram, the donor nucleus, taking over the control of its development into differentiated animal cells. Thus, here we hypothesize that it is possible to reprogram a fully differentiated animal cell nucleus by transferring the nucleus to an enucleated protoplast (Fig. 3).

Conclusions and Future Perspectives

It is known that every cell in an adult individual, either animal or plant, possess a complete set of genes with genetic and biochemical potential, and under appropriate conditions these cells are able to dedifferentiate. However, only plants have the ability to regenerate complete individuals from one single isolated somatic cell.[207,208] Therefore, plants have higher dedifferentiation plasticity and capacity than animals, and utilizing these features of plants may create new avenues for research and treatment of diseases. The fully differentiated plant cells can be isolated from the original tissue by removing the cell wall, resulting in protoplasts, in which repressed genes reactivate and encode molecules needed for initiation of the developmental processes. Several studies have shown that plants

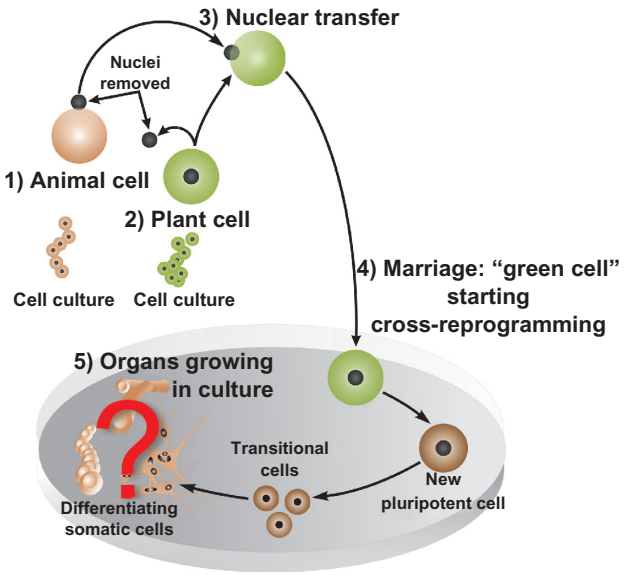

Figure 3. Model for reprogramming of an animal cell nucleus by transfer into enucleated protoplast. Animal cell nucleus from differentiated animal cell transferred (1) into the enucleated protoplast. In the resulting cell, nuclear reprogramming (2) takes place, resulting in totipotent cells, which under controlled conditions differentiate into a pluripotent cell known as the "green cell". The green cell can then differentiate (3) into any cell types that are identical in genetic makeup to the donor animal cell.

and animals use conserved epigenetic mechanisms to regulate gene expression, but many different enzymes catalyze the same mechanisms.[137]

Thus, the main question to be answered in the future is why is complete regeneration is possible in plants, but not in animals. One possibility is that the epigenetic apparatus of plants is able to "open up", whereas that of the animals cannot, due to its more "rigid" chromatin structure preventing the reactivation of repressed regions. However, it remains unclear whether plants have less "rigid" chromatin structure than animals or if they can utilize their epigenetic apparatus more efficiently for gene reactivation. These exciting issues need to be solved by future studies. As a further possibility for utilizing epigenetic mechanisms, more efficient methods can be designed to treat diseases that are currently incurable, such as the vast majority of cancers or neural disorders, such as Parkinson's and Alzheimer diseases. However, diet, nutrition, and exercise also influence or control epigenetic mechanisms, and these may be used better to treat diseases such as obesity and cancer. In other areas, such as agriculture, preventing of crops from infections, or enhancing plants to adapt to stress and climate change, modification of epigenetic mechanisms could also be a target for future investigations.

In conclusion, an increased understanding of the details of epigenetic regulation of gene's function and the use of more signaling models for detecting factors involved in regulating epigenetic processes may provide the potential to generate reprogrammed pluri- or totipotent cells without the use of cancer genes or egg cells. These developments will help to design better treatments for human diseases by using the power of epigenetic factors and mechanisms.

Author Contributions

Wrote the first draft of the manuscript: IS, ZN, AK. Contributed to the writing of the manuscript: IS, ZN, GH, RM, GS, AK. Agree with manuscript results and conclusions: IS, ZN, GH, RM, GS, AK. Jointly developed the structure and arguments for the paper: IS, ZN, GH, RM, GS, AK. Made critical revisions and approved final version: IS, ZN, GH, RM, GS, AK. All authors reviewed and approved of the final manuscript.

Funding

Supported by: SROP-4.2.2.A-11/1/KONV-2012-0024 SROP-4.2.2.A-11/1/KONV-2012-0017, Nat. Sci. Res. Fund.-OTKA K7159 and American Heart Association Founders Aff., 0555897T.

Acknowledgment

We thank Tibor A. Rauch for critical reading of the manuscript.

Competing Interests

Author(s) disclose no potential conflicts of interest.

Disclosures and Ethics

As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests.

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