View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by IntechOpen

We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

4,800
Open access books available

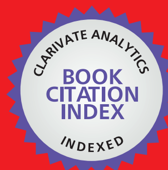
122,000
International authors and editors

135M
Downloads

154
Countries delivered to

Our authors are among the
TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

14

Illuminating Hidden Features of Stem Cells

Gideon Grafi[1*], Rivka Ofir[3], Vered Chalifa-Caspi[2] and Inbar Plaschkes[2]
[1]French Associates Institute for Agriculture and Biotechnology of Drylands, Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev,
[2]The National Institute for Biotechnology, Ben-Gurion University of the Negev,
[3]Ben Gurion University of the Negev and Dead Sea & Arava Science Center, Hazeva Branch,
Israel

1. Introduction

In recent years stem cells become a major topic both publicly and scientifically owing to their promise to cure diseases and restore organ functionality. Yet our understanding of the biology of stem cells and their inherent features is largely lagging behind the great promise of using these cells in transplantation therapies. In this chapter we highlight several aspects of the biology of stem cells/induced pluripotent stem (iPS) cells that are often overlooked. We define stem cells not only by their developmental capacities, namely, self-renewal and multi-lineage differentiation, but also by their inherent features. We present new bioinformatic data and draw the stem cell picture based on recent knowledge emerging from studying stem cells in plant and animal systems with emphasis on chromatin structure. We highlight some of the potentially hazardous pathways associated with cellular dedifferentiation (iPS cells) and with culturing stem cells. Notably, genomic modification associated with iPS cells is often discussed with respect to the methodology of introducing reprogramming genes into the host cells, namely, lentiviral/retroviral transduction, while ignoring the potential genomic modification naturally associated with dedifferentiation or with stress events. Based on our understanding of cellular processes accompanied stress response and cellular dedifferentiation we discuss strategies for improving the quantity and quality of iPS cells.

2. Inherent features of stem cells

2.1 What are stem cells?

One major, long recognized, pitfall in stem cell biology is that stem cells are commonly defined by their developmental potentialities, namely, self-renewal and multitype differentiation, rather than by their intrinsic features (Potten & Loeffler, 1990; McKay, 2000). This often leads to the erroneous assumption that reentry of stem cells to the cell cycle for the purpose of the so-called 'self-renewal' represents an inherent feature of stem cells. Consequently, the term stem cell culture has been established, leading biologists to incorrectly assume that stem cell features can be fully maintained under culture conditions (Grafi & Avivi, 2004). Considering that animal somatic cells are capable to become stem cells *via* dedifferentiation (Kurisaki et al., 2010), the traditional developmental capacity-based

definition of stem cells may be useless in distinguishing between genuine stem cells and somatic cells that have the capacity to become stem cells. Obviously, defining stem cells by their inherent features or signature rather than their developmental capabilities is necessary. The signature of a given differentiated cell is commonly reflected in its gene expression profile. The attempts to uncover the 'stem cell signature' or the 'stemness genes' via transcriptome analysis of different stem cell culture lines were failed as these experiments yielded different 'signatures' and non-overlapping 'stemness genes' (Ramalho-Santos et al., 2002; Ivanova et al., 2002; Fortunel et al., 2003). This again highlighted the problem of defining stem cells by their developmental capacity, namely, self-renewal (cultured cells) (Grafi & Avivi, 2004). Contrary to the idea that stem cells represent a unique entity that is characterized by the expression of specific set of 'stemness' genes, it has been suggested that stem cells represent a unique transient state characterized by promiscuous expression of marker genes (Zipori, 2004). Consequently, several possibilities could describe the transcriptional landscapes of stem cells: (i) differentiation/lineage-specific genes are not expressed in stem cells, (ii) differentiation/lineage-specific genes are widely expressed in stem cells but at a very low level, and (iii) differentiation/lineage-specific genes are not expressed but assume a transcriptionally competent chromatin state (Zipori, 2004; Meshorer & Misteli, 2006; Efroni et al., 2008). The later is supported by the fact that stem cells, like dedifferentiating cells, acquire open, decondensed chromatin architecture, which is essential though not sufficient for initiating gene transcription (Williams et al., 2003; Grafi, 2004; Gaspar-Maia et al., 2011) as well as by the finding that some non-expressed genes or genes expressed at low levels in embryonic stem (ES) cells are primed but their transcription is attenuated by a unique combination of permissive and restrictive chromatin marks (Azuara et al., 2006; Bernstein et al., 2006). Apparently open chromatin configuration appears to be an intrinsic feature of stem cells that can be used to distinguish between actual stem cells and potential stem cells.

2.2 Chromatin structure and regulation in brief

The basic structural unit of chromatin is the nucleosome, which is composed of DNA wrapped around histone octamer made of two of each of core histone proteins, namely, H2A, H2B, H3 and H4. All core histone proteins share a common structural motif called the histone fold consisting of three alpha helices connected by short loops (Arents & Moudrianakis, 1995). The X-ray crystal structure of the nucleosome core particle showed that interactions between core histone proteins or between histone octamer and duplex DNA are largely dependent on the histone fold motif. The histone amino-terminal tail is unstructured and protruding outside the nucleosomal disk where it can contact with neighboring nucleosomes or with proteins or protein complexes that affect chromatin structure and function. The structure of chromatin is highly dynamic, facilitating the transition between permissive and repressive chromatin. This dynamic structure is controlled by multiple types of reversible chemical modifications that occur on the DNA (cytosine methylation) or on the DNA interacting core histone proteins. Most modifications of histone proteins occur on the N-terminal tails and include acetylation, methylation, phosphorylation, and ubiquitination. These chemical modifications can directly affect the interaction with DNA or generate binding sites for recruitment of proteins or protein complexes that affect the structure and function of chromatin and consequently differentiation and development. Gene promoters can be found in three fundamental states determined by their histone modification marks, namely, restrictive (e.g., methylated

H3K9/K27), permissive (e.g., methylated H3K4, acetylated H3K9) or both restrictive and permissive (e.g., methylated H3K27, methylated H3K4, also known as 'bivalent' state). For example, SET domain-containing histone methyltransferase proteins, such as Clr4 (Cryptic locus regulator) in *Schizosaccharomyces pombe* and SUV39H1 and SUV39H2 in humans are enzymes that methylate histone H3 specifically at lysine 9 (Rea et al., 2000). This methylation generates a binding site for Heterochromatin protein 1 (HP1) (Bannister et al., 2001; Lachner et al., 2001), which is required for the establishment of a condensed, repressive chromatin. Conversely, histone acetylation or methylation of histone H3 at lysine 4 are often associated with 'open' chromatin configuration and gene transcription (Eberharter & Becker, 2002; Lee & Workman, 2007; Eissenberg & Shilatifard, 2010). More recently it has been shown that some non-expressed genes or genes expressed at low levels in ES cells carry both permissive (H3K4me3; trimethylated H3K4) and restrictive (H3K27me3; trimethylated H3K27) chromatin marks (Azuara et al., 2006; Bernstein et al., 2006). This unique 'bivalent' chromatin state suggests a model in which many tissue-specific regulatory genes are 'primed' but their transcription is delayed until entry into a specific differentiation pathway that dictate either activation (e.g., recruitment of H3K27 demethylases) or silencing (e.g., recruitment of H3K4 demethylses) of the gene locus (Lan et al., 2008). Notably, bivalent domains were found to occur also in the rice genome in somatic cells where a large proportion of genes possessing the repressive mark H3K27me3 also contained permissive marks of H3K4me3 and H3K9ac (acetylated H3K9) (He et al., 2010).

2.3 Chromatin modifiers and the establishment of stem cell state

Stem cells as well as dedifferentiating cells have been shown to maintain their chromatin in an open, decondensed configuration relative to differentiated cells (reviewed in Gaspar-Maia et al., 2011). Accordingly, chromatin decondensation appears to be a hallmark of cells engaged in switching fate, a process, which requires the acquisition of a stem cell-like state, which is essential for normal development. For example, during fertilization, decondensation of sperm chromatin is essential for the formation of the male pronucleus, which is necessary for successful fertilization (Longo & Anderson, 1968). Interestingly, sperm chromatin decondensation can be mimicked in *Xenopus* egg extracts and requires nucleoplasmin, an acidic, thermostable, abundant nuclear protein capable of binding histone proteins (Laskey et al., 1978; Philpott et al., 1991; Katagiri & Ohsumi, 1994). Similarly, *Xenopus* egg extracts can induce chromatin decondensation of heterologous somatic nuclei. It has been shown that nuclei derived from chicken erythrocytes incubated in *Xenopus* egg extract were induced to replicate their DNA, a process preceded by two sequential phases of chromatin decondensation (Blank et al., 1992). Chromatin decondensation was reported in cells of regenerating tissues of the tubeworm *Owenia fusiformis* 12 h after amputation (Fontes et al., 1980), a stage, which might correlate with cellular dedifferentiation whereby somatic cells at the amputation site withdraw from their differentiated state and acquire stem cell-like state (Grafi, 2004). In plants, chromatin decondensation was found to be associated with cellular dedifferentiation induced following exposure to various stress conditions (e.g., protoplasting, exposure to dark) (Zhao et al., 2001; Tessadori et al., 2007; Damri et al., 2009, Grafi et al., 2011).

The evidence that stem cells may assume a more open chromatin conformation is dated about 40 years ago with the description of the morphological characteristics of the so-called stem cells in the bone marrow by Murphy et al. (1971). Using electron microscopy examination of 3-day post hypoxic marrow, they found that the large mononuclear cells are

characterized by a fine nuclear chromatin (leptochromatic) with some peripheral condensation. Likewise, electron microscopy inspection of the erythropoietic cells of the chick showed that stem cells are characterized by large nuclei and homogenous euchromatin, while maturation is accompanied by an increase in nuclear condensation indicated by areas of heterochromatin (MacRae & Meetz, 1970). Also, ultrastructure of developing erythrocytes in the bone marrow of human adults showed that in the earliest stages, the nucleoplasm was chiefly composed of euchromatin, while during maturation heterochromatin was rapidly increased concomitantly with a reduction in nuclear size (Miura et al., 1974). Furthermore, using micrococcal nuclease, Weintraub (1978) showed that the nucleosome repeat length (NRL) increases from 190 to 212 basepairs during erythropoiesis in the chick, which was accompanied by a significant increase in the concentration of red cell specific histone H5, a linker histone necessary for stabilization of higher order chromatin structure (Robinson & Rhodes, 2006). This increase in NRL has recently been shown to be required for the formation of the 30 nm DNA fiber-induced chromatin compaction (Routh et al., 2008). Notably, similarly to animal stem cells, ultrastructural observations of the nuclei in the shoot apex (the plant stem cell niche) of the plant *Tradescantia paludosa* showed that a large proportion of the chromatin is organized as less condensed, diffused euchromatin fibrils (Booker & Dwivedi, 1973).

It has been demonstrated that chromatin decondensation of somatic nuclei in *Xenopus* egg extracts requires the chromatin-remodeling nucleosomal adenosine triphosphatase (ATPase) ISWI (Kikyo et al., 2000). More recently, it has been shown that embryonic stem cells are extensively engaged in global gene transcription, due in part to overrepresentation of chromatin-remodeling genes and the general transcription machinery; this activity is diminished upon differentiation (Efroni et al., 2008). Knockdown by RNAi of the SWI/SNF remodeling factor BRG1 and of the ISWI-related chromodomain helicase DNA binding protein 1-like (Chd1l) resulted in impairment of ES cell differentiation and proliferation (Efroni et al., 2008). Indeed, knockdown of Chd1 in ES cells established its importance for maintaining open chromatin, pluripotency of embryonic stem cells, as well as for reprogramming of somatic cell to pluripotent state (Gaspar-Maia et al., 2009). Furthermore, reprogramming of somatic cells by the expression of the four transcription factors, Oct4, Sox2, Klf4 and c-Myc is facilitated by combined expression of chromatin remodeling components of the BAF (Brg1/Brahma-associated factor) complex (Singhal et al., 2010).

2.4 Pattern of chromatin modifier gene expression in stem cells and dedifferentiating cells

Open chromatin configuration emerges as a fundamental theme in pluripotent stem cell biology, and should be primarily served as a major attribute for defining the stem cell state. This open chromatin conformation is crucial for maintaining the stemness state, which might confer stem cells with the capacity for rapid switching into the appropriate transcriptional program upon induction of differentiation and with the flexibility needed for differentiation into multiple cell types. Thus, in looking for molecular features defining stem cells we should highlight the molecular components regulating chromatin structure, namely chromatin modifier genes (CMGs). There are two possible ways for maintaining the unique chromatin state characteristic of stem cells, which we define as quantitative and qualitative approaches. The quantitative approach suggests that the flexibility needed for stem cells to differentiate into multiple cell types can be achieved by promiscuous expression of CMGs, while the qualitative approach suggests that the open chromatin conformation characteristic

of stem cells is maintained by selective expression and repression of genes that promote the formation of permissive and restrictive chromatin, respectively.

The quantitative approach gains support from analysis of the gene expression profiles of the plant shoot apical meristem (SAM) stem cells (Yadav et al., 2009). By introducing fluorescent reporters into SAM-enriched *Arabidopsis* background line, Yadav et al. (2009) have isolated by FACS three distinct cell types of the SAM stem cell niche and analyzed the transcriptome profiles of these cells. One major attribute of SAM stem cells appears to be a flexible chromatin state demonstrated by the overrepresentation of genes involved in chromatin organization (Yadav et al., 2009). Remarkably, further analysis of the available microarray dataset of the SAM stem cells revealed that among the 445 CMGs, which were represented on the ATH1 array, stem cells showed the expression of 297 genes whose expression signal is higher than 256 (>2^8). This unusual expression of chromatin modifier genes in SAM stem cells might confer the flexibility required for maintaining the pluripotent state of stem cells.

Apparently, this widespread expression of CMGs observed in SAM stem cells is not mimicked in dedifferentiating plant cells. Transcriptome profiling of dedifferentiating protoplast cells (Damri et al., 2009) showed that among the 465 CMGs, which were represented on the ATH1 array only 95 genes displayed an expression signal higher than 256 (>2^8). However, close examination of these CMGs highlighted the qualitative approach revealing that many of the genes that were down-regulated in dedifferentiating protoplasts, such as histone deacetylase encoding genes SRT2, HDA14 and HDT4, as well as linker histone genes Hon1 and Hon2 are implicated in chromatin compaction, while many of the CMGs that were up-regulated, such as histone acetyltransferase encoding genes HAF1, HAC5 and HAG3 as well as histone demethylase genes JMJ21 and JMJ13 are implicated in chromatin decondensation. Thus the expression profile of CMGs favors the acquisition of an open chromatin conformation in dedifferentiating cells.

Since open chromatin configuration characterizes stem cells as well as dedifferentiated cells in plants and animals, the study of these cells in plants might have bearing to understanding inherent biological features of animal stem cells.

2.5 CMGs as a tool for monitoring pluripotency

We hypothesized that these features of quantitative and qualitative CMG expression of SAM stem cells and dedifferentiating cells, respectively, might hold also for human stem cells and dedifferentiating cells and could serve as a distinguishing feature between animal cells displaying various levels of pluripotency. To assess the qualitative approach, we first predefined a set of human CMG families, which were selected based on their predicted chromatin function, namely, histone deacetylases (HDACs) involved in heterochromatin formation, histone acetyltransferases (HATs), which are involved in euchromatin formation and the jumonji family of histone demethylases implicated in both euchromatin and heterochromatin formation. We then checked the expression level of these predefined CMGs (listed in Table 1) in a recently published meta-analysis of human gene expression datasets (Lukk et al., 2010). In this study, the authors collected and integrated high-quality data from 5,372 samples from 206 different studies, all generated using Affymetrix U133A arrays and deposited in public databases. Using text mining and curation, the authors binned the samples in 369 biological groups, each representing a particular cell or tissue type, disease state or cell line. Additionally, samples were grouped more crudely according to different criteria, one of which divided the samples into four meta-groups: Cell lines, Neoplasms,

Non-neoplastic diseases, and Normal. We have downloaded the entire gene expression matrix from the ArrayExpress repository (accession number E-MTAB-62), and reduced it to include only the 1033 samples of the "Normal" meta-group, and the 40 CMGs indicated in Table 1. Subsequently, probe sets which represented the same gene were consolidated as follows: (1) probe sets with signal intensity lower than 5 (in log2 scale) in all 1033 samples were removed, provided that at least one probe set for the same gene had signal intensity higher than 5 (in log2 scale) in at least one of the samples. (2) The signal intensities of the remaining probe sets were averaged to yield a single expression value per gene per sample. The resulting matrix was loaded into Partek Genomics Suite™. To allow for gene-wise comparison of expression profiles, expression values were standardized such that the mean and standard deviation of each gene's values were 0 and 1, respectively. The standardized dataset was subjected to bi-directional hierarchical clustering using Euclidean distance and complete linkage. Finally, sample annotations indicating 6 meta-groups (brain, cell line, hematopoietic system, incompletely differentiated, muscle, solid tissue) and operating laboratory were added. As shown in Fig. 1, based on their expression pattern, the CMGs were clearly clustered into three major clades. Most intriguing is clade 1 displaying

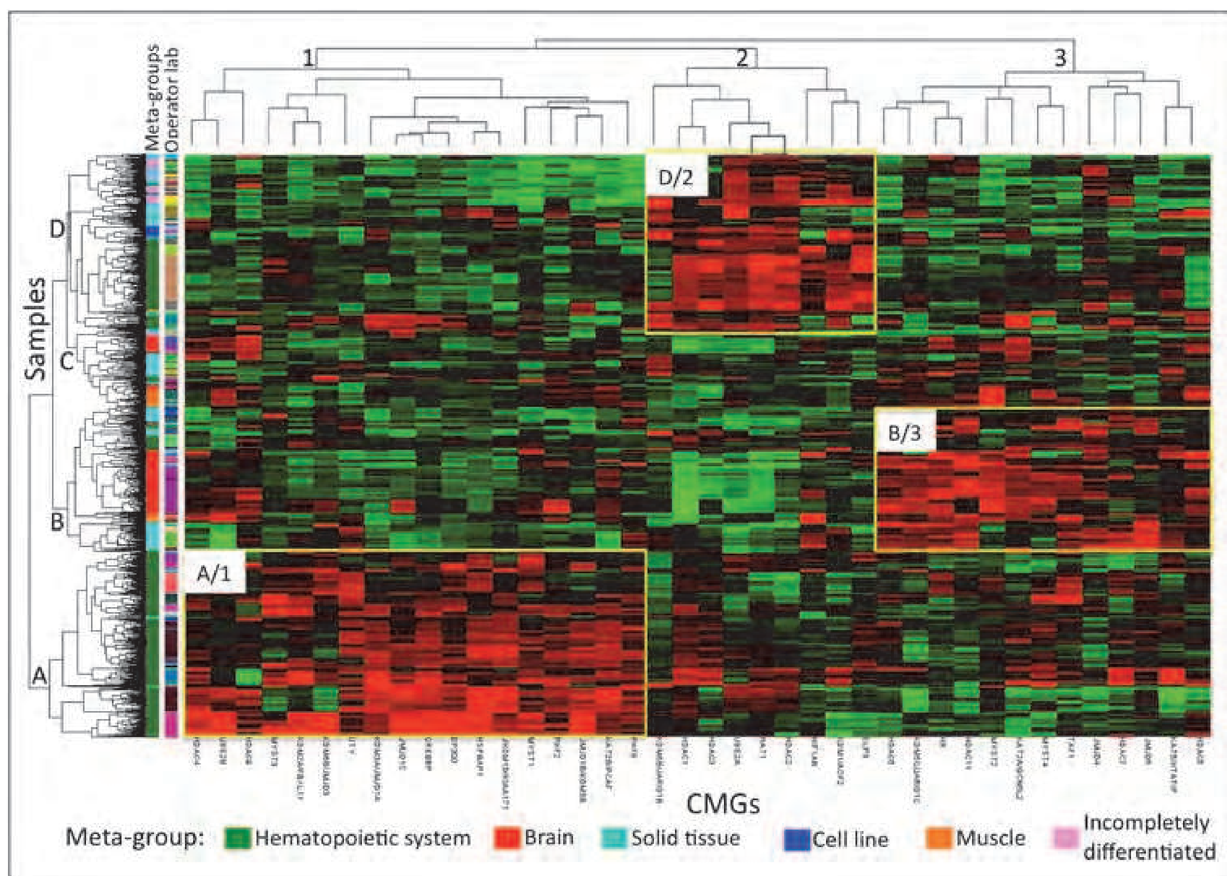


Fig. 1. Bi-directional hierarchical clustering of 40 CMGs expression in 1033 normal human tissues derived from public datasets compiled by Lukk et al. (2010). Gene-wise standardized expression level is shown as colored squares on a red-black-green scale (from up- to down-regulation, respectively). Samples are labeled by two color columns indicating their meta-group and operating laboratory. The three clustered CMG groups (1-3) displaying peculiar upregulation in a specific sample clade (A, B, D) are boxed yellow.

upregulation of 18 CMGs out of the 40 examined as they form a homogenous cluster of samples (clade A in Fig. 1) derived almost entirely from the hematopoietic system reported by various labs. Notably, 14 out of the 18 CMGs in clade 1 are implicated in euchromatin formation and gene activation (Table 1). Interestingly, 10 out of the 14 CMGs belong to the jumonji protein family of histone demethylases, most of which (8 genes) are implicated in removal of repressive methyl group and formation of euchromatin (Table 1). In clade 2, 9 CMGs are upregulated, most of which are implicated in heterochromatin formation (Table 1). This group defines a cluster (clade D in Fig. 1) composed of samples derived from the hematopoietic system (mostly differentiated lymphocytes, macrophages and granulocytes), cell line, solid tissue and incompletely differentiated cells. Clade 3 is characterized by the expression of 13 CMGs distributed almost equally between genes implicated in eu- and heterochromatin and defines a cluster (clade B in Fig. 1) composed of diverse samples derived mostly from the brain but also from hematopoietic system, muscle and solid tissue. Based on this analysis and the capacity for open chromatin configuration (Table 1) we predict that clade A represents a group of cells most of which from the hematopoietic system with the highest pluripotency level, clade B has moderate and clade D has the lowest pluripotency level. Clade C generates a cluster of cells derived from the brain, solid tissue and muscle with no clear clustering of the CMGs.

To evaluate the strength of our prediction we analyzed the expression pattern of the predefined CMGs in a microarray dataset (GSE18290) obtained for early stages of human embryos, which include one-, two-, four-, and eight-cell stage embryos, morula stage and the blastocyst stage (Xie et al., 2010). The raw CEL files of the respective Affymetrix Human Genome U133 Plus 2.0 Arrays were downloaded from NCBI GEO and further preprocessed using RMA in Partek Genomics Suite™. Expression values of 38 out of the 40 predefined CMGs were standardized and subjected to gene wise hierarchical clustering using Euclidean distance and complete linkage. The resulting heat map shown in Fig. 2 revealed a sharp transition in the expression of CMGs between four- and eight-cell embryos, which might reflect transition from high pluripotency level (totipotency) to a more reduced one. The finding that blastomers derived from two- and four-cell embryos retain totipotency (Van de Velde et al., 2008) may support this notion. The abrupt transition in pattern of CMG expression is consistent with the largest changes in transcriptome profile observed between four- and eight-cell stages of human embryos (Xie et al., 2010) as well as with the timing of zygote genome activation (ZGA) in humans, which commences between the four- and eight-cell stages of embryo development concomitantly with the degradation of maternal transcripts (Braude et al., 1988; Nothias et al., 1995). Based on the expression pattern of CMGs, cell samples could be divided into two major groups, namely group A, which is composed of one-to four-cell embryos displaying 18 (probably maternal) CMGs (clade 2), most of which (12 out of 18) are implicated in euchromatin formation and gene transcription. Group B is composed of eight-cell embryos, morula and blastocyst displaying upregulation of 20 CMGs (probably zygotic transcripts, clade 1) distributed equally between CMGs implicated in eu- and heterochromatin formation. Based on this analysis, it appears that overrepresentation of CMGs whose products are associated with open chromatin and gene transcription characterizes cells with high pluripotency level and thus supporting our hypothesis. We suggest that this attribute may faithfully serve as a tool for assessing the pluripotency level of the cell.

Gene name	Gene function	Predicted chromatin function	Group A/1	Group B/3	Group D/2
HDAC4	Histone deacetylase	**Hetero**	+	-	-
UBE2B	H2B ubiquitination	**Eu**	+	-	-
HDAC9	Histone deacetylase	**Hetero**	+	-	-
MYST3	HAT	**Eu**	+	-	-
KDM2A	H3K36 demethylase	**Hetero**	+	-	-
KDM6B	H3K27 demethylase	**Eu**	+	-	-
UTY	H3K27 demethylase	**Eu**	+	-	-
KDM3A	H3K9 demethylase	**Eu**	+	-	-
JMJD1C	H3K9 demethylase	**Eu**	+	-	-
CREBBP	HAT	**Eu**	+	-	-
EP300	HAT	**Eu**	+	-	-
HSPBAP1	Jumonji protein	unknown	+	-	-
JHDM1D	H3K9/K27 demethyl.	**Eu**	+	-	-
MYST1	HAT	**Eu**	+	-	-
PHF2	H3K9 demethylase	**Eu**	+	-	-
KAT2B	HAT	**Eu**	+	-	-
KDM3B	H3K9 demethylase	**Eu**	+	-	-
PHF8	H3K9/K27 demethyl.	**Eu**	+	-	-
KDM5B	H3K4 demethylase	**Hetero**	-	-	+
HDAC1	Histone deacetylase	**Hetero**	-	-	+
HDAC3	Histone deacetylase	**Hetero**	-	-	+
UBE2A	H2B ubiquitination	**Eu**	-	-	+
HAT1	Cytoplasmic HAT	unknown	-	-	+
HDAC2	Histone deacetylase	**Hetero**	-	-	+
HIF1AN	Jmj protein	**Hetero?**	-	-	+
KDM1	H3K4 demethylase	**Hetero**	-	-	+
ELP3	HAT	**Eu**	-	-	+
HDAC6	Histone deacetylase	**Hetero**	-	+	-
KDM5C	H3K4 demethylase	**Hetero**	-	+	-
HR	Jumonji protein	unknown	-	+	-
HDAC11	Histone deacetylase	**Hetero**	-	+	-
MYST2	HAT	**Eu**	-	+	-
KAT2A	HAT	**Eu**	-	+	-
MYST4	HAT	**Eu**	-	+	-
TAF1	HAT	**Eu**	-	+	-
JMJD4	unknown	unknown	-	+	-
HDAC7	Histone deacetylase	**Hetero**	-	+	-
KDM8	H3K36 demethylase	**Hetero**	-	+	-
KAT5	HAT	**Eu**	-	+	-
HDAC5	Histone deacetylase	**Hetero**	-	+	-

Table 1. Summary of the expression pattern of CMGs in the different clustered groups (shown in Fig. 1) and their predicted chromatin function. Eu, Euchromatin, HAT, histone acetyltransferase; Hetero, heterochromatin

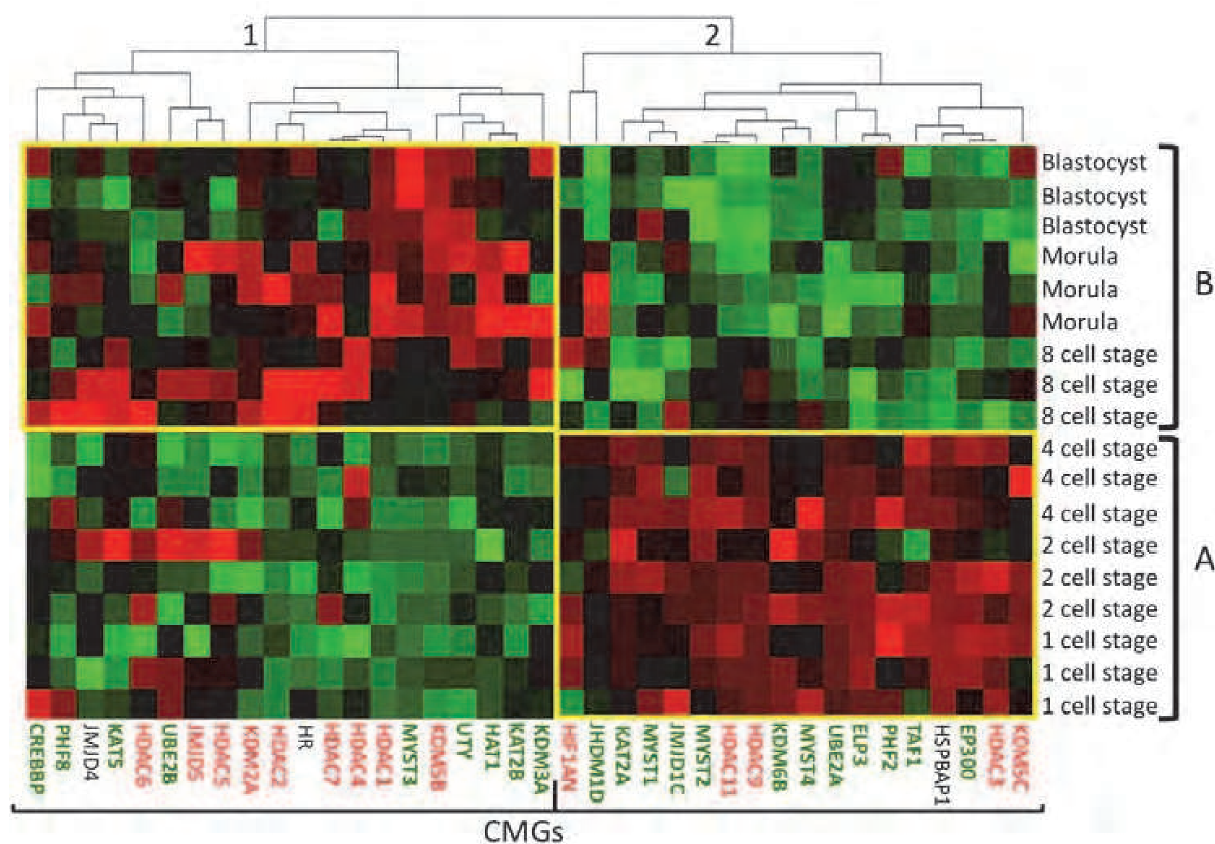


Fig. 2. Clustering of 38 CMGs expression in human embryos at various developmental stages derived from public datasets compiled by Xie et al. (2010, GSE18290). Gene-wise standardized expression level is shown as colored squares on a red-black-green scale (from up- to down-regulation, respectively). The two clustered CMG groups (upper dendrogram) displaying peculiar upregulation in a specific sample group (A and B) are boxed yellow. CMGs highlighted green and red are predicted to be involved in formation of euchromatin and heterochromatin, respectively.

3. Hazardous pathways associated with cellular dedifferentiation (iPS cells) and with culturing stem cells

It is now quite accepted that reprogramming of somatic cells in culture as well as culturing of stem cells are prone to hazardous genomic modifications and thus undermine their potential use in regenerative medicine. The complexity of hES cells is highlighted by the fact that injection of these cells (in their un-transformed state) into immunodeficient mice induced teratoma formation - a practice commonly used as a stringent assay to prove their existence. Indeed, animal embryonic stem cells often displayed genomic abnormalities in culture, which frequently resulted in malignant transformation (Lefort et al. 2009; Ben David & Benvenisty, 2011). Similarly, human iPS cells (Takahashi & Yamanaka, 2006), like human ES cells can acquire genetic abnormalities in the culture (Mayshar et al. 2010). These genetic aberrations are presumed to arise in part from culture adaptation while others are suspected to originate from the parent somatic cell. The later may be overcome by derivation of somatic cells from embryonic tissue (Ben-David et al., 2010). Yet, derivation of somatic cells from embryos, such as mouse embryonic fibroblasts (MEFs) did not improve significantly

reprogramming efficiency or reduced genetic and epigenetic abnormalities (Marión et al., 2009). Cell type of origin has a major effect on the properties of mouse iPS cells at early-passages, where iPS cells retain a transient epigenetic memory of their somatic cells of origin demonstrated by their differential gene expression and differentiation capacity, while at late passages these differences were attenuated (Polo et al., 2010).

Reprogramming of somatic cells to generate iPS cells is believed to commence with the introduction of the so-called 'pluripotent genes' into cultured somatic cells. Commonly, researchers are not aware that reprogramming has already initiated in somatic cells when they are removed from the *soma* and placed in tissue culture environments. Cell culturing of primary cells might impose an extreme stress over the cells resulting in reprogramming, dedifferentiation and acquisition of pluripotency prior to reentry of somatic cells [e.g., mouse embryonic fibroblasts (MEFs)] to the cell cycle (Fig. 3) – a stage, which is often overlooked. This aspect of cell culturing has been highlighted by Barbra McClintock (1984) in her Nobel article 'The significance of responses of the genome to challenge'. McClintock (1984) has recognized the potential for hazardous genetic variation that can be induced following exposure of cells to stress (e.g., cell culturing, virus infection) stating "Some responses to stress are especially significant for illustrating how a genome may modify itself when confronted with unfamiliar conditions. Changes induced in genomes when cells are removed from their normal locations and placed in tissue culture surroundings are outstanding examples of this. The establishment of a successful tissue culture from animal cells, such as those of rat or mouse, is accompanied by readily observed genomic restructuring." McClintock predicted that these aberrant genome responses to stress are likely to be induced by mobilization of transposable elements. Therefore, we should consider the possibility that the genotype(s) of somatic cells entering the cell cycle may not be identical to the genotype of the original somatic cells; a genotype(s) conferring increasing fitness for tissue culture conditions may prevail.

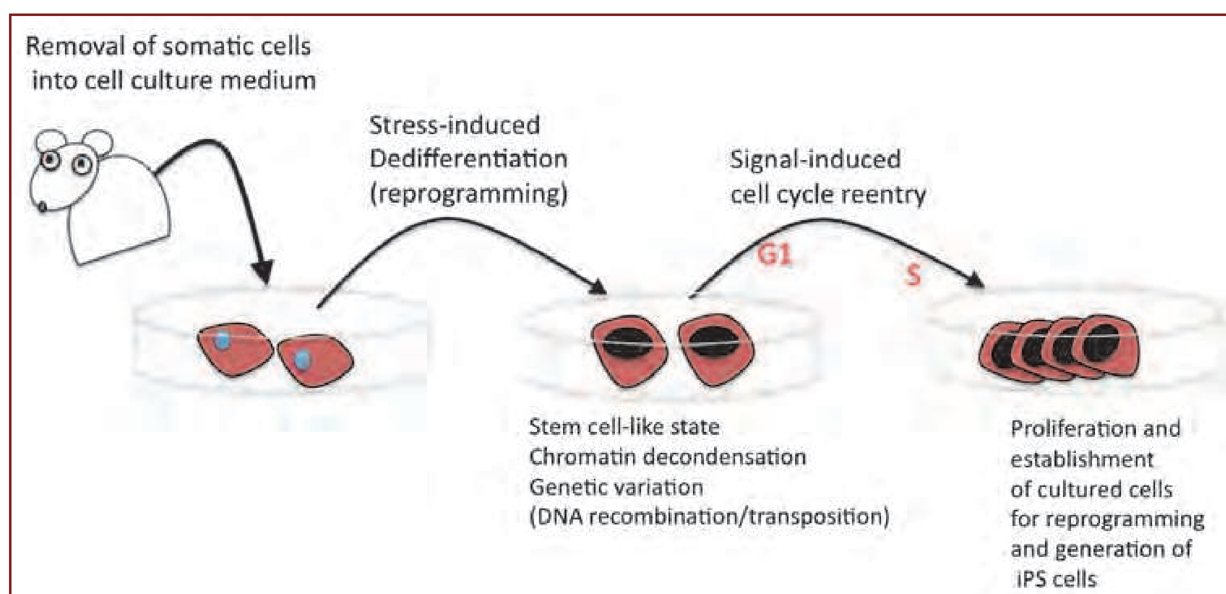


Fig. 3. Reprogramming of somatic cells. The removal of somatic cells from the body is stressful and might induce dedifferentiation (reprogramming) and acquisition of stem cell-like state prior to reentry into the cell cycle (G1-S transition). Note that due to genetic variation induced during dedifferentiation the genotype of cells entering the cell cycle may be different from the genotype of the original somatic cells.

In animals, somatic cell nuclear transfer (SCNT) as well as iPSCs, both are dedifferentiation-driven processes appear to be very limited (Tamada & Kikyo, 2004; William & Plath, 2008). This is probably due, at least in part, to a high frequency of p53-mediated DNA damage response and p53-dependent apoptosis, which are acting to ensure genomic integrity (Marión et al., 2009). Although, it is presumed that failure in reprogramming is selective for those cells with preexisting DNA damage, it cannot be excluded that DNA damage or 'irreversible genomic modifications' are induced in cells (e.g., protoplasts, primary MEFs) in the course of stress-induced cellular dedifferentiation (Fig. 3) or cell culturing via DNA recombination and DNA transposition (Pouteau et al., 1991; Hirochika, 1993; Grafi et al., 2007; Grafi, 2009; Grafi et al., 2011). Almost half of the human genome is composed of transposable elements, some of which still retain their capacity for transposition into genes, which could lead to genetic instability and human diseases (reviewed in Belancio et al. 2009). Indeed, Alu and Line-1 expression and retrotransposition have been reported in human ES cells and in human neural progenitor cells (Garcia-Perez et al., 2007; Coufal et al., 2009; Macia et al., 2011). Notably, some of these retroelements are often activated in various cell lines following exposure to stress (reviewed in Oliver & Greene, 2009).
Presently, it is not clear why the genome become vulnerable under certain stress conditions. One possibility is that following exposure to stress the genome is reacting by extensive and stochastic reorganization culminating in global chromatin decondensation and acquisition of dedifferentiated, stem cell-like state (reviewed in Grafi, 2009). Stochastic epigenetic modifications may release constraints over transposable elements resulting in their activation and transposition into other chromosomal sites. Stress-induced decrease in methylation of repetitive elements [long interspersed nucleotide element (LINE)-1 and Alu repetitive elements] was reported in blood samples derived from elderly individuals following exposure to traffic particles (Baccarelli et al., 2009). A whole-genome profiles of DNA methylation of several human iPS cell lines, showed aberrant reprogramming of DNA methylation; regions proximal to centromeres and telomeres display incomplete reprogramming of non-CG methylation, and differences in CG methylation and histone modifications (Lister et al., 2011).

4. Discussion

The capacity of somatic cells to dedifferentiate and acquire stem cell-like state is an important goal toward developing an efficient tool for use in regenerative medicine. Yet, the process of dedifferentiation is complex and unsafe resulting from increased incidents of DNA transposition/recombination-induced genetic variation and genome instability (Grafi, 2009). In addition, the findings that both embryonic stem cells (ESCs) and iPS cells show higher frequencies for genetic abnormalities relative to other cell lines limit their suitability for clinical use in regenerative medicine (Mayshar et al., 2010; Laurent et al., 2011). The generation of iPS cells from somatic cells by various means emerged as one reasonable approach for generating autologous iPS cells for clinical applications. This methodology solves the problem of transplant rejection and also moral concerns often raised regarding the use of ES cells. However, the process of generating iPS cells is very inefficient, has very low rate of success and may be subjected to hazardous genetic variation (Grafi, 2009) leading, at least partly, to p53-dependent cell death (Marión et al., 2009). The limited success in iPS cells is enigmatic inasmuch as dedifferentiation is an integral process of development both in plants and animals. It underlies the regenerative capacity of certain vertebrates

(Brockes & Kumar, 2008) as well as of plants (reviewed in Grafi, 2004) and, at least partly, the capacity for transdifferentiation, that is, the conversion of one cell type to another (Tosh & Slack, 2002).

We should consider the fact that the capacity for switching cell fate in animals suggests that the machinery needed for this transition is already exist in the framework of the cell including the pluripotent genes (e.g., OCT4, SOX2) whose ectopic expression in cultured cells may induce formation of pluripotent stem cells. Hence, why introducing these genes exogenously instead of activating the endogenous ones? Accumulating data suggest that reprogramming does not require the 'four factors' (OCT4, SOX2, KLF4, c-Myc) and can be carried out with only one factor OCT4 (Kim et al., 2009). This raises the question whether reprogramming is achieved due to ectopic expression of pluripotent genes or due to endogenous ones being activated as a consequence of the procedure itself (viral transduction), namely, virus-induced chromatin reorganization (Monier et al., 2000). Indeed, induction of pluripotency does not necessarily require exogenous factors and can be carried out by extracts derived from stem cells or undifferentiated human NCCIT carcinoma cells (Taranger et al., 2005). More recently it has been shown that pretreatment of somatic cells with chromatin modulators, namely, DNA methyltransferase and histone deacetylase inhibitors can improve reprogramming and the formation of hESC-like colonies by embryonic stem cell extracts (Han et al., 2010). Chromatin architecture is a fundamental theme in pluripotency and as such should be the primary means for activation of silent genes whose products involved in establishing of the pluripotent state. Support to this view are the open chromatin configuration characteristic of stem cells and the finding that chromatin remodeling factors play a critical role in maintaining open chromatin and reprogramming of somatic cells to pluripotent state (Efroni et al., 2008; Gaspar-Maia et al., 2009). Accordingly, it has recently been shown that chromatin-remodeling components of the BAF complex facilitate the reprogramming of somatic cells into pluripotent state (Singhal et al., 2010).

5. Conclusions

The use of CMGs for reprogramming of somatic cells has not been exploited sufficiently. We should consider manipulating of the activities of chromatin modifiers capable of facilitating the formation of open chromatin conformation. Emphasis should be given to those genes whose products actively remove repressive marks from histone tails, such as histone demethylases (Table 1), as a necessary step toward opening of otherwise closed chromatin. In this respect, together with existing tools such as the PluriTest (Muller et al., 2011), our bioinformatic data suggest that the transcription profile of CMGs can be formulated into a robust bioinformatic tool for assessing the pluripotency level of cells.

Obviously, the removal of cells (e.g., stem cells, somatic cells) from their normal location in the body and placing them under tissue culture conditions is hazardous and should be avoided, unless we find the way(s) to control the extent of genetic variation induced during dedifferentiation and cell culturing. An alternative approach has been suggested by Abramovich et al. (2008) in their article entitled 'Have we reached the point for in vivo rejuvenation?' The authors suggested to try and imitate natural rejuvenation processes and to test the possibility of inducing dedifferentiation and the pluripotent state in somatic cells *in vivo.* Future challenges will be to gain knowledge and find the appropriate means for inducing dedifferentiation at specific tissue or organ *in vivo* for efficient and safe regenerative medicine.

6. Acknowledgements

This chapter is dedicated to the memory of Dr. Amir Abramovich, a brilliant scientist who passed away untimely and who was so enthusiastic about the potential use of the process of dedifferentiation for the purpose of *in vivo* rejuvenation.
We thank Eitan Rubin for bioinformatics discussions and Vadim Fraifeld for critical reading of the chapter. This work was supported by The Israel Science Foundation (ISF) grant No. 476/09 to G.G. and V.C.-C.

7. References

Abramovich, A., Muradian, K.K. & Fraifeld, V.E. (2008). Have we reached the point for in vivo rejuvenation? *Rejuvenation Res* 11:489-492.

Arents, G. & Moudrianakis, E.N. (1995). The histone fold: a ubiquitous architectural motif utilized in DNA compaction and protein dimerization. *Proc Natl Acad Sci U S A.* 92:11170-11174.

Azuara, V., Perry, P., Sauer, S., Spivakov, M., Jørgensen, H.F., John, R.M., Gouti, M., Casanova, M., Warnes, G., Merkenschlager, M. & Fisher, A.G. (2006). Chromatin signatures of pluripotent cell lines. *Nat Cell Biol* 8:532-538.

Baccarelli, A., Wright, R.O., Bollati, V., Tarantini, L., Litonjua, A.A., Suh, H.H., Zanobetti, A., Sparrow, D., Vokonas, P.S. & Schwartz, J. (2009). Rapid DNA methylation changes after exposure to traffic particles. *Am J Respir Crit Care Med* 179:572-578.

Bannister, A.J., Zegerman, P., Partridge, J.F., Miska, E.A., Thomas, J.O., Allshire, R.C. & Kouzarides, T. (2001). Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. *Nature* 410:120-124.

Belancio, V.P., Deininger, P.L. & Roy-Engel, A.M. (2009). LINE dancing in the human genome: transposable elements and disease. Genome Medicine 1:97.

Ben-David, U., Benvenisty, N. & Mayshar, Y. (2010). Genetic instability in human induced pluripotent stem cells: classification of causes and possible safeguards. *Cell Cycle* 9:4603-4604.

Ben-David, U. & Benvenisty, N. (2011). The tumorigenicity of human embryonic and induced pluripotent stem cells. *Nat Rev Cancer* 11:268-277.

Bernstein, B.E., Mikkelsen, T.S., Xie, X., Kamal, M., Huebert, D.J., Cuff, J., Fry, B., Meissner, A., Wernig, M., Plath, K., Jaenisch, R., Wagschal, A., Feil, R., Schreiber, S.L. & Lander, E.S. (2006). A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell* 125: 315-326.

Blank, T., Trendelenburg, M. & Kleinschmidt, J. (1992). Reactivation of DNA replication in erythrocyte nuclei by Xenopus egg extract involves energy-dependent chromatin decondensation and changes in histone phosphorylation. *Exp Cell Res* 202:224-232.

Booker, C.E. & Dwivedi, R.S. (1973). Ultrastructure of meristematic cells of dormant and released buds in Tradescantia paludosa. *Exp Cell Res* 82:255-261.

Brockes, J.P. & Kumar, A. (2008). Comparative aspects of animal regeneration. *Annu Rev Cell Dev Biol.* 24:525-249.

Braude, P., Bolton, V. & Moore, S. (1988). Human gene expression first occurs between the four-and eight-cell stages of preimplantation development. *Nature* 332:459–461.

Coufal, N.G., Garcia-Perez, J.L., Peng, G.E., Yeo, G.W., Mu, Y., Lovci, M.T., Morell, M., O'Shea, K.S., Moran, J.V. & Gage, F.H. (2009). L1 retrotransposition in human neural progenitor cells. *Nature* 460:1127-1131.

Damri, M., Ben-Meir, H., Avivi, Y., Caspi-Chalifa, V., Wolfson, M., Fraifeld, V. & Grafi, G. (2009). Senescing cells share common features with dedifferentiating cells. *Rejuvenation Research* 12:435-443.

Eberharter, A. & Becker, P.B. (2002). Histone acetylation: a switch between repressive and permissive chromatin. Second in review series on chromatin dynamics. *EMBO Rep* 3:224-229.

Efroni, S., Duttagupta, R., Cheng, J., Dehghani, H., Hoeppner, D.J., Dash, C., Bazett-Jones, D.P., Le Grice, S., McKay, R.D., Buetow, K.H., Gingeras, T.R., Misteli, T. & Meshorer, E. (2008). Global transcription in pluripotent embryonic stem cells. *Cell Stem Cell* 2:437-447.

Eissenberg, J.C. & Shilatifard, A. (2010). Histone H3 lysine 4 (H3K4) methylation in development and differentiation. *Dev Biol* 339: 240-249.

Fontes, M., Marilley, M., Le Parco, Y. & Thouveny, Y. (1980). Variations in accessibility of DNA during traumatic regeneration by Owenia fusiformis (polychaete annelid). *Cell Differ* 9:83-93

Fortunel, N.O., Out, H.H., Ng, H.H., Chen, J., Mu, X., Chevassut, T., Li, X., Joseph, M., Bailey, C., Hatzfeld, J.A., Hatzfeld, A., Usta, F., Vega, V.B., Long. P.M., Libermann, T.A. & Lim, B. (2003). Comment on " 'Stemness': transcriptional profiling of embryonic and adult stem cells" and "a stem cell molecular signature". *Science* 302:393.

Garcia-Perez, J.L., Marchetto, M.C., Muotri, A.R., Coufal, N.G., Gage, F.H., O'Shea, K.S. & Moran JV. (2007). LINE-1 retrotransposition in human embryonic stem cells. *Hum Mol Genet* 16:1569-1577.

Gaspar-Maia, A., Alajem, A., Meshorer, E. & Ramalho-Santos, M. (2011). Open chromatin in pluripotency and reprogramming. *Nat Rev Mol Cell Biol* 12:36-47.

Grafi, G. & Avivi, Y. (2004). Stem cells: a lesson from dedifferentiation. *Trends Biotech* 22:388-389.

Grafi, G. (2004). How cells dedifferentiated: a lesson from plants. *Dev Biol* 268:1-6

Grafi, G., Ben-Meir, H., Avivi, Y., Moshe, M., Dahan, Y. & Zemach, A. (2007). Histone methylation controls telomerase-independent telomere lengthening in cells undergoing dedifferentiation. *Dev Biol* 306:838-846.

Grafi, G. (2009). The complexity of cellular dedifferentiation: implications for regenerative medicine. *Trends Biotech* 27:329-332.

Grafi, G., Chalifa-Caspi, V., Nagar, T., Plaschkes, I., Barak, S. & Ransbotyn, V. (2011). Plant response to stress meets dedifferentiation. *Planta* 233:433–438.

Han, J., Sachdev, P.S. & Sidhu, K.S. (2010). A combined epigenetic and non-genetic approach for reprogramming human somatic cells. *PLoS One* 5:e12297.

He, G., Zhu, X., Elling, A.A., Chen, L., Wang, X., Guo, L., Liang, M., He, H., Zhang, H., Chen, F., Qi, Y., Chen, R. & Deng, X.W. (2010). Global epigenetic and transcriptional trends among two rice subspecies and their reciprocal hybrids. *Plant Cell* 22:17-33.

Hirochika, H. (1993). Activation of tobacco retrotransposons during tissue culture. *EMBO J.* 12:2521-2528.

Ivanova, N.B., Dimos, J.T., Schaniel, C., Hackney, J.A., Moore, K.A & Lemischka, I.R. (2002). A stem cell molecular signature. *Science* 298:601-604.

Katagiri, C. & Ohsumi, K. (1994). Remodeling of sperm chromatin induced in egg extracts of amphibians. *Int J Dev Biol* 38: 209-216.

Kikyo, N., Wade, P.A., Guschin, D., Ge, H. & Wolffe, A.P. (2000). Active remodeling of somatic nuclei in egg cytoplasm by the nucleosomal ATPase ISWI. *Science* 289:2360-2362.

Kim, J.B., Greber, B., Arau´zo-Bravo, M.J., Meyer, J., Park, K.I., Zaehres, H. & Schöler, H.R. (2009). Direct reprogramming of human neural stem cells by OCT4. *Nature* 461, 649–653.

Kurisaki, A., Ito, Y., Onuma, Y., Intoh, A. & Asashima, M. (2010). In vitro organogenesis using multipotent cells. *Hum Cell* 23:1-14.

Lachner, M., O'Carroll, D., Rea, S., Mechtler, K. & Jenuwein, T. (2001). Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. *Nature* 410:116-120.

Lan, F., Nottke, A.C. & Shi, Y. (2008). Mechanisms involved in the regulation of histone lysine demethylases. *Curr Opin Cell Biol* 20:316-325.

Laskey, R.A., Honda, B.M., Mills, A.D. & Finch, J.T. (1978). Nucleosomes are assembled by an acidic protein which binds histones and transfers them to DNA. *Nature* 275:416-420.

Laurent, L.C., Ulitsky, I., Slavin, I., Tran, H., Schork, A., Morey, R., Lynch, C., Harness, J.V., Lee, S., Barrero, M.J., Ku, S., Martynova, M., Semechkin, R., Galat, V., Gottesfeld, J., Izpisua Belmonte, J.C., Murry, C., Keirstead, H.S., Park, H.S., Schmidt, U., Laslett, A.L., Muller, F.J., Nievergelt, C.M., Shamir, R. & Loring, J.F. (2011). Dynamic Changes in the Copy Number of Pluripotency and Cell Proliferation Genes in Human ESCs and iPSCs during Reprogramming and Time in Culture. *Cell Stem Cell* 8:106-118.

Lee, K.K. & Workman J.L. (2007). Histone acetyltransferase complexes: one size doesn't fit all, *Nat. Rev. Mol. Cell Biol.* 8:284–295.

Lefort, N., Perrier, A.L., Laâbi, Y., Varela, C. & Peschanski, M. (2009). Human embryonic stem cells and genomic instability. *Regen Med* 4:899-909.

Lister, R., Pelizzola, M., Kida, Y.S., Hawkins, R.D., Nery, J.R., Hon, G., Antosiewicz-Bourget, J., O'Malley, R., Castanon, R., Klugman, S., Downes, M., Yu, R., Stewart, R., Ren, B., Thomson, J.A., Evans, R.M. & Ecker, J.R. (2011). Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. *Nature* 471:68-73.

Longo, F.J. & Anderson, E. (1968). The fine structure of pronuclear development and fusion in the sea urchin, *Arbacia punctulata*. J *Cell Biol* 39:339-368.

Lukk, M., Kapushesky, M., Nikkilä, J., Parkinson, H., Goncalves, A., Huber, W., Ukkonen, E. & Brazma, A. (2010). A global map of human gene expression. *Nat Biotechnol* 28:322-324.

Macia, A., Muñoz-Lopez, M., Cortes, J.L., Hastings, R.K., Morell, S., Lucena-Aguilar, G., Marchal, J.A., Badge, R.M. & Garcia-Perez, J.L. (2011). Epigenetic control of

retrotransposon expression in human embryonic stem cells. *Mol Cell Biol* 31:300-316.

MacRae, E.K. & Meetz, G.D. (1970). Electron microscopy of the ammoniacal silver reaction for histones in the erythropoietic cells of the chick. *J Cell Biol* 45:235-245.

Marión R.M., Strati K., Li H., Murga M., Blanco R., Ortega S., Fernandez-Capetillo O., Serrano M. & Blasco M.A. (2009). A p53-mediated DNA damage response limits reprogramming to ensure iPS cell genomic integrity. *Nature* 460:1149-1153.

Mayshar, Y., Ben-David, U., Lavon, N., Biancotti, J.C., Yakir, B., Clark, A.T., Plath, K., Lowry, W.E. & Benvenisty, N. (2010). Identification and classification of chromosomal aberrations in human induced pluripotent stem cells. *Cell Stem Cell* 7:521-531.

McClintock, B. (1984). The significance of responses of the genome to challenge. *Science* 226:792-801.

McKay, R. (2000). Stem cells—hype and hope. *Nature* 406:361–364.

Meshorer, E. & Misteli, T. (2006). Chromatin in pluripotent embryonic stem cells and differentiation. *Nat Rev Mol Cell Biol* 7:540-546.

Miura, A.B., Shibata, A., Akihama, T., Endo, Y. & Saito, Y. (1974). Ultrastructure of developing erythrocytes. *Tohoku J Exp Med* 112:299-313.

Monier, K., Armas, J.C., Etteldorf, S., Ghazal, P. & Sullivan, K.F. (2000). Annexation of the interchromosomal space during viral infection. *Nat Cell Biol* 2:661– 665.

Müller, F.J., Schuldt, B.M., Williams, R., Mason, D., Altun, G., Papapetrou, E.P., Danner, S., Goldmann, J.E., Herbst, A., Schmidt, N.O., Aldenhoff, J.B., Laurent, L.C. & Loring, J.F. (2011). A bioinformatic assay for pluripotency in human cells. *Nat Methods* 8:315-317.

Murphy, M.J. Jr., Bertles, J.F. & Gordon, A.S. (1971). Identifying characteristics of the haematopoietic precursor cell. *J Cell Sci* 9:23-47.

Nothias, J.Y., Majumder, S., Kaneko, K.J. & DePamphilis, M.L. (1995). Regulation of gene expression at the beginning of mammalian development. *J Biol Chem* 270:22077-22080.

Oliver, K.R. & Greene, W.K. (2009) Transposable elements: powerful facilitators of evolution. *BioEssays* 31:703-714.

Philpott, A., Leno, G.H. & Laskey, R.A. (1991). Sperm decondensation in Xenopus egg cytoplasm is mediated by nucleoplasmin. *Cell* 65:569-578.

Polo, J.M., Liu, S., Figueroa, M.E., Kulalert, W., Eminli, S., Tan, K.Y., Apostolou, E., Stadtfeld, M., Li, Y., Shioda. T., Natesan, S., Wagers, A.J., Melnick, A., Evans, T. & Hochedlinger, K. (2010). Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. *Nat Biotechnol* 28:848-855.

Potten, C.S. & Loeffler, M. (1990). Stem cells: attributes, cycles, spirals, pitfalls and uncertainties. Lessons for and from the crypt. *Development* 110:1001-1020.

Pouteau, S., Huttnerl, E., Grandbastien, M.A. & Caboche, M. (1991). Specific expression of the tobacco Tntl retrotransposon
in protoplasts. *EMBO J.* 10:1911-1918.

Ramalho-Santos, M., Yoon, S., Matsuzaki, Y., Mulligan, R.C. & Melton, D.A. (2002). "Stemness": transcriptional profiling of embryonic and adult stem cells. *Science* 298:597-600.

Rea, S., Eisenhaber, F., O'Carroll, D., Strahl, B.D., Sun, Z.W., Schmid, M., Opravil, S., Mechtler, K., Ponting, C.P., Allis, C.D. & Jenuwein, T. (2000). Regulation of chromatin structure by site specific histone H3 methyltransferases. *Nature* 406:593-599.

Robinson, P.J. & Rhodes, D. (2006). Structure of the '30 nm' chromatin fibre: a key role for the linker histone. *Curr Opin Struct Biol* 16:336-343.

Routh, A., Sandin, S. & Rhodes, D. (2008). Nucleosome repeat length and linker histone stoichiometry determine chromatin fiber structure. *Proc Natl Acad Sci* U S A. 105:8872-8877.

Singhal, N., Graumann, J., Wu, G., Araúzo-Bravo, M.J., Han, D.W., Greber, B., Gentile, L., Mann, M. & Schöler, H.R. (2010). Chromatin-Remodeling Components of the BAF Complex Facilitate Reprogramming. *Cell* 141:943-955.

Takahashi, K. & Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126:663-676.

Tamada, H. & Kikyo, N. (2004). Nuclear reprogramming in mammalian somatic cell nuclear cloning. *Cytogenet Genome Res* 105:285–291.

Taranger, C.K., Noer, A., Sørensen, A.L., Håkelien, A.M., Boquest, A.C. & Collas, P. (2005). Induction of dedifferentiation, genome wide transcriptional programming, and epigenetic reprogramming by extracts of carcinoma and embryonic stem cells. *Mol Biol Cell* 16:5719-5735.

Tessadori, F., Chupeau, M.C., Chupeau, Y., Knip, M., Germann, S., van Driel, R., Fransz, P. & Gaudin, V. (2007). Large-scale dissociation and sequential reassembly of pericentric heterochromatin in dedifferentiated Arabidopsis cells. *J Cell Sci* 120:1200-1208.

Tosh, D. & Slack, J.M. (2002). How cells change their phenotype. *Nat Rev Mol Cell Biol* 3:187-194.

Van de Velde, H., Cauffman, G., Tournaye, H., Devroey, P. & Liebaers, I. (2008). The four blastomeres of a 4-cell stage human embryo are able to develop individually into blastocysts with inner cell mass and trophectoderm. *Hum Reprod* 23:1742-1747.

Weintraub, H. (1978). The nucleosome repeat length increases during erythropoiesis in the chick. *Nucleic Acids Res* 5:1179-1188.

William, E.L. & Plath, K. (2008). The many ways to make an iPS cell. *Nature Biotechnol* 26:1246-1248.

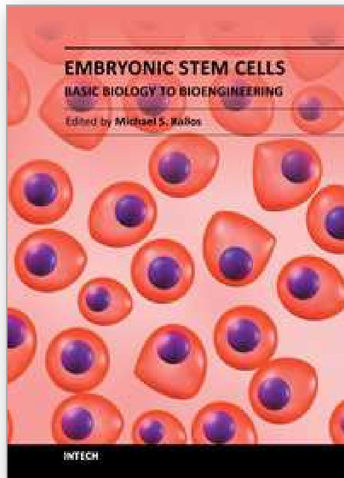
Williams, L., Zhao, J., Morozova, N., Li, Y., Avivi, Y. & Grafi, G. (2003). Chromatin reorganization accompanying cellular dedifferentiation is associated with modifications of histone H3, redistribution of HP1, and activation of E2F-target genes. *Dev Dyn* 228:113-120.

Xie, D., Chen, C.C., Ptaszek, L.M., Xiao, S., Cao, X., Fang, F., Ng, H.H., Lewin, H.A., Cowan, C. & Zhong, S. (2010). Rewirable gene regulatory networks in the preimplantation embryonic development of three mammalian species. *Genome Res* 20:804-815.

Yadav, R.K., Girke, T., Pasala, S., Xie, M. & Reddy, G.V. (2009). Gene expression map of the Arabidopsis shoot apical meristem stem cell niche. *Proc Natl Acad Sci* U S A. 106:4941-4946.

Zhao, J., Morozova, N., Williams, L., Libs, L., Avivi, Y. & Grafi, G. (2001). Two phases of chromatin decondensation during dedifferentiation of plant cells: Distinction between competence for cell fate switch and a commitment for S phase. *J Biol Chem* 276:22772-22778.

Zipori, D. (2004). The nature of stem cells: state rather than entity. *Nat Rev Genet* 5:873-878.



Embryonic Stem Cells - Basic Biology to Bioengineering
Edited by Prof. Michael Kallos

ISBN 978-953-307-278-4
Hard cover, 478 pages
Publisher InTech
Published online 15, September, 2011
Published in print edition September, 2011

Embryonic stem cells are one of the key building blocks of the emerging multidisciplinary field of regenerative medicine, and discoveries and new technology related to embryonic stem cells are being made at an ever increasing rate. This book provides a snapshot of some of the research occurring across a wide range of areas related to embryonic stem cells, including new methods, tools and technologies; new understandings about the molecular biology and pluripotency of these cells; as well as new uses for and sources of embryonic stem cells. The book will serve as a valuable resource for engineers, scientists, and clinicians as well as students in a wide range of disciplines.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Gideon Grafi, Rivka Ofir, Vered Chalifa-Caspi and Inbar Plaschkes (2011). Illuminating Hidden Features of Stem Cells, Embryonic Stem Cells - Basic Biology to Bioengineering, Prof. Michael Kallos (Ed.), ISBN: 978-953-307-278-4, InTech, Available from: http://www.intechopen.com/books/embryonic-stem-cells-basic-biology-to-bioengineering/illuminating-hidden-features-of-stem-cells

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike-3.0 License, which permits use, distribution and reproduction for non-commercial purposes, provided the original is properly cited and derivative works building on this content are distributed under the same license.