UDC 606:577.21 https://doi.org/10.15407/biotech10.01.017

MOLECULAR MECHANISMS OF PLURIPOTENCY INDUCTION AND REPROGRAMMING OF SOMATIC CELLS

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Received 07.11.2016

To analyze the recent studies elucidating the molecular mechanisms of pluripotency induction and crucial stages of the reprogramming process was the aim of the review. The key focus is on the factors enabling switch between the reprogramming stages. It is concluded that one of the key barriers for IPSC applications is the multi-stage nature of somatic cells reprogramming that features both stochastic early phases and deterministic establishment of pluripotent regulatory network. Despite thousands of scientific studies, various reprogramming protocols restrict effective research analysis and identification of molecular reprogramming mechanisms. In order to specify accurate reprogramming algorithms and develop more effective protocols of patient specific reprogrammed cells cultivation, the future researches require focus on the phase transition switchers.

Key words: pluripotency induction, reprogramming, somatic cells, induced pluripotent stem cells.

Despite the tremendous potential of embryonic stem cells (ESCs) application in medicine, diagnostics and biotechnology researches, these studies faced numerous scientific, methodological and ethical barriers for decades. However, totipotency (ability to form all the cells in the body and the whole embryo) and immortality (unlimited cell divisions) as unique ESC features allured scientists with highly promising perspective applications in transplantology, immunology, gerontology and pharmacology for experimental and therapeutic purposes. Within early embryonic development cells lose the both characteristics, as in 5–6 days upon zygote formation the differentiation mechanisms start functioning in the cells. The specialization process subsequently provides for the terminally differentiated cells. There are few stem cells (SCs) in adult humans which is too limited to provide for cellular regeneration in cases of significant traumas and lesions [1–3]. Nevertheless, studies in the field of SC generation and use found numerous constraints included restricted SC sources and their disadvantages, immunologic incompatibility of SCs and recipient's tissues, ethical considerations, etc.

Over 50 years ago the scientific society informed about the ECS phenomenon and overcoming Weisman's barrier (the term was coined in the end of 19th century). It's worth mentioning that German researcher August Weisman theorized that the somatic cell state can not be changed due to finite inactivation of "useless" genetic code during differentiation. In 1962 British biologist John Gurdon became the first to overcome Weisman's barrier and reprogram somatic cell with nuclear transfer to enucleated ovule. The experiment produced embryo being genetically identical to the donor of somatic cell [4, 5]. 1981 Sir Martin Evans, Matthew Kaufmann and Gail R. Martin revolutionized the ontogenetic world with discovery of ESCs derived from mouse embryos and tremendous characteristics of these cells [6, 7]. In 1998 research team headed by James Thompson cultivated embryonic stem cell lines from human blastocysts [8]. Decades were taken to research ways of transdifferentiation (fate conversion of terminally differentiated cells) via heterokaryons and fusion with other cell types [9].

10 years ago a breakthrough reprogramming and SC cultivation approach was reported in the publication on pluripotency induction by introducing transcription factors (TFs). In 2006 Japan scientists Takahashi and Jamanaka reported successful generation of induced pluripotent SCs with 4 TFs (transcriptional factors) that nowadays are referred as Yamanaka factors. As distinct from totipotent SC, induced pluripotent stem cells (iPSCs) can give rise to any cell types excluding extraembryonic cells and feature with several epigenetic differences being under active discussion in specialized media for SCs, molecular biology and genetics. In the famous publication cited all over the thousands of articles in pluripotency induction Takahashi and Jamanaka highlighted the experiment to test the hypothesized pluripotency induction with:

i) TFs crucial for the pluripotency maintenance in ESCs — OCT3/4, SOX2, NANOG;

ii) genes overexpressed in tumours and specific for maintenance ESC characteristics and proliferation — B-catenin, C-MYC, E-RAS, KLF4, STAT3;

iii) other ESC-specific genes.

Having successfully derived iPSC from mouse fibroblasts by introducing all 24 candidate genes and TFs, the researchers tested consecutively various TF-combinations. The experiment resulted in the identification of baseline TF cocktail required to reprogram differentiated cells and induce pluripotency. Yamanaka reprogramming cocktail consisted of OCT3/4, KLF4, SOX2, C-MYC was reported in the mentioned publications [10, 11].

Comparing with the cell reprogramming techniques described above (namely somatic cell nuclear transfer and somatic cell fusion) the pluripotency induction opened a flexible and attractive transdifferentiation paradigm. The pluripotency induction approach discovered by Yamanaka and Takahashi opened a new source of patient specific SCs and promised wide applications in regenerative and cell therapy as well as removed methodological and ethical hurdles from SC usage in pharmacology, toxicology and ontological studies. In the field of modern cell technologies the breakthrough created methodological background for ongoing initiatives in somatic cells transdifferentiation. Targeted transdifferentiation of patient specific somatic cells is now widely used in pharmacological studies and regenerative medicine [2, 8–11]. The next decade demonstrated intense iPSC researches and numerous successful experiments of pluripotency induction with other TF combinations e.g. NANOG and LIN28 or NR5a2, SOX1, ESRRB and GLIS-1 [12].

Despite strong research focus on somatic cell reprogramming and transdifferentiation processes, molecular and epigenetic mechanisms of cellular repgrogramming mediated by Yamanaka TFs or other reprogramming cocktails remained unclear. Poor understanding of these algorithms hinders new effective reprogramming techniques to be developed and restricts biotechnological potential of iPSCs as well as patient specific SC cultivation perspectives [13]. Here we provide a brief review of key epigenetic processes and phase switchers in reprogramming reported recently according to the expression data of reprogrammed cells [9–13].

Key stages of somatic cells reprogramming and pluripotency induction

Explicit ontological and epigenetic data on embryonic development processes the mammal zygote shows to develop milliards of highly specialized cells *in vivo* don't explain the somatic cell reprogramming mechanisms *in vitro*. TF-induced reprogramming requires about 2–3 weeks depending on protocol and methods of TF delivery. The process suffers from rather low efficiency (~1%) [14]. However, valuable information on TF interplay and molecular changes in cells can be sourced from studies of cellular reprogrammed intermediates and screening of partially reprogrammed cells (pre-iPSCs).

Expression analysis and morphological studies run over reprogramming timeline demonstrate stochastic and deterministic stages of pluripotency induction.

The initial stochastic stage also referred as early or partial reprogramming [9, 15] features with the following:

- dependence on ectopic (out of place, norm) expression of reprogramming TF;
- poorly predictable or stochastic nature;
- morphological changes of reprogrammed cells;
- increased TF-mediated proliferation.

Dependence on ectopic expression supposes that TF removal on this stage causes the reprogramming cell state to return to the initial one. This could be explained with unstable and reversible nature of TF-induced epigenetic modifications (e.g. emerging activating chromatin modifications — H3 lysine 4 methylation due to C-MYC).

The first reprogramming stage is widely recognized as stochastic as the stage durability varies significantly even within one protocol. For the fact the researchers account multiple molecular and epigenetic routes that responsible for somatic identity genes are inactivated and pluripotency genes are derepressed [15–17]. These processes result in morphological alterations (incl. reduced cell size) and increased proliferation [12, 17].

The next and final deterministic stage of pluripotency induction is a kind of bottleneck in reprogramming technique thus showing relatively low efficiency. Over 90% of transdifferentiated cells remain partially reprogrammed and do not acquire functional pluripotency.

Upon profound investigation of this problem the researchers developed the elite model of reprogramming process. According to the model the deterministic stage is entered by only few partially reprogrammed somatic cells that belong to limited SC progenitors. These cells are competent for pluripotency induction and endogenous SOX2 expression [15]. However, recent analytical studies ruled out the hypothesis and proved the ability to convert terminally differentiated somatic cells into iPSCs [9, 16, 18].

In contrast to stochastic stage the deterministic one possesses the following characteristics:

- the reprogrammed cells do not depend on the ectopic TF expression;
- silencing of the ectopic TF expression is a crucial factor to determine transition of the reprogrammed cells to this stage;
- the present stage results in formation of successfully reprogrammed iPSCs expressing pluripotency markers, showing self-renewal and being able to create embryos [19].

Each of the reprogramming stages consists of several phases. At the same time the scientific publications differ in referring specific events to certain phases of pluripotency induction. The phase structure below covers the epigenetic and molecular reprogramming mechanisms and grounds on conceptual views of reprogramming pioneers and prevalent scientific approaches in Europe, Asia and USA [9, 18, 20, 21].

Here we are ging to highlight the sequence of key events specific for the phases during stochastic and deterministic reprogramming stages. There are also several factors specified as phase transition drivers according to the recent studies and research materials.

Stochastic dedifferentiation of somatic cell

The TF-induced reprogramming of somatic cells starts with the morphological and epigenetic alterations mentioned above. Initial changes of chromatin state stimulate reduced cell sizes and partial loss of somatic markers as well as increases proliferation. Within the present phase TFs act on accessible sites of the expressed genes and regions with absent or unstable histone modifications responsible for somatic epigenome [22]. Therefore TF activity disrupts transcription regulatory network of genes encoding somatic phenotype and initiates chromatin remodeling.

Chromatin remodeling supposes relocation or regrouping of nucleosomes in response to:

- ATP-dependent protein complexes;
- histone methylation or acetylation.

Thus TF-induced chromatin remodeling processes cause gene activation or repression [18, 23, 24]. Firstly, TFs increase active genes chromatin density due to interaction with trimethylated histone H3 lysine 39 and dimethylated histone H3 lysine 79 (H3K36me3 and H3K79me2, respectively). Secondly, TFs influence promotors with trimethylated histone H3 lysine 4 (H3K4me3) specific for the proliferation genes in fibroblasts [18].

The genes silenced during cellular differentiation are reactivated due to H3K27me3 demethylation with lysine-specific demethylase 6A (KDM6A) as well. Israel geneticists and microbiologists reported in the research findings that KDM6A induced removal of repressive marks is a key factor that determines efficiency of early reprogramming in mouse and human somatic cells [25]. KDM6A directly interacts with reprogramming TFs and catalyzes demethylation of repressive histone modifications H3K27me3 thus contributing to initial pluripotency induction. Recent experimental studies demonstrate that KDM6A knockout fibroblast lines failed to induce pluripotency. Therefore KDM6A ensures demethylation of repressive chromatin marks and derepresses pluripotency genes. Among these we can mention SALL 1 and 4, undifferentiated embryonic cell transcription factor 1 (UTF1) allowing for cellular independency on ectopic TF expression during the later reprogramming phases [26, 27].

Subsequently, initial dedifferentiation of fibroblasts accumulates activating histone modifications (also due to KDM6A activity)

and removes some repressive marks the somatic cells acquired during differentiation in order to ensure somatic epigenome stability. These non-specific alterations of the fibroblast eu- and heterochromatin contribute to induction of some pluripotency genes.

Early stochastic reprogramming phase

During the initial stochastic reprogramming of fibroblasts, TF activities that were described above are accompanied by the changes in chromatin state and cellular metabolism; also there's an increase in the number of markers that are not inherent to somatic cells, as well as loss of somatic identity markers in the transcriptional profile of said cells.

During this phase of pluripotency induction, activated are the proliferative epigenetic patterns which were active on early stages of ontogenesis and are considered especially sensitive to TFs. In particular, there is a further strengthening of the expression of abovementioned genes responsible for cell proliferation. Meanwhile, at this phase of reprogramming the proliferation level is above the characteristic parameters for somatic cells [9, 15, 28].

In addition to enhanced proliferation, during this phase, the influence of phosphoinositide 3-kinase (PI3K)/protein kinase Akt signaling pathways activates the expression of C-MYC and NANOG [16]. These events switch cell's metabolism from oxidative phosphorylation to glycolysis [29]. *In vivo* this ESC metabolism happens due to adaptation to hypoxic conditions of early embryonic development. Correspondingly, the introduction of hypoxia during the pluripotency induction of somatic cells *in vitro* increases the efficiency of reprogramming, stimulating the activation of the metabolism typical for ESCs [30]. It is worth noting that this exact metabolism is inherent to cancer cells — also under the effect of C-MYC — and also to SCs of mature organisms.

The defining processes that occur during the initial reprogramming of fibroblasts include mesenchymal-to-epithelial transition (MET). During MET occurs the inactivation of genes of somatic cells which are responsible for the mesenchymal phenotype, and the reprogrammed cells acquire epithelial phenotype and characteristics that were inherent to ESCs *in vitro* but were lost in the early stages of ontogenesis (Fig. 1).

As you remember, *in vivo* the epithelial-to-mesenchymal transition (EMT) happens during the embryonic development affected by zinc-finger TF (SNAIL1, SNAIL2, ZEB1 and 2) as well as transforming growth factor β (TGFβ), and is accompanied by decreased expression of E-cadherin. MET, as an event reversed to EMT, is a derepression of epithelial program and is primarily provided by:

- suppressed expression of EMT inductor zinc-finger TF Snail, affected by SOX2;
- stimulation of expression of E-cadherin due to Klf4;
- inactivation of TGFβ receptors due to C-MYC [31].

It is worth noting that numerous research groups report on the induction of MET occurring in mouse and human somatic cells in only due to inhibition of TGFβ, suggesting that suppression of TGFβ and interaction of reprogramming TFs with bone morphogenic proteins are MET's primary causes [30, 13].

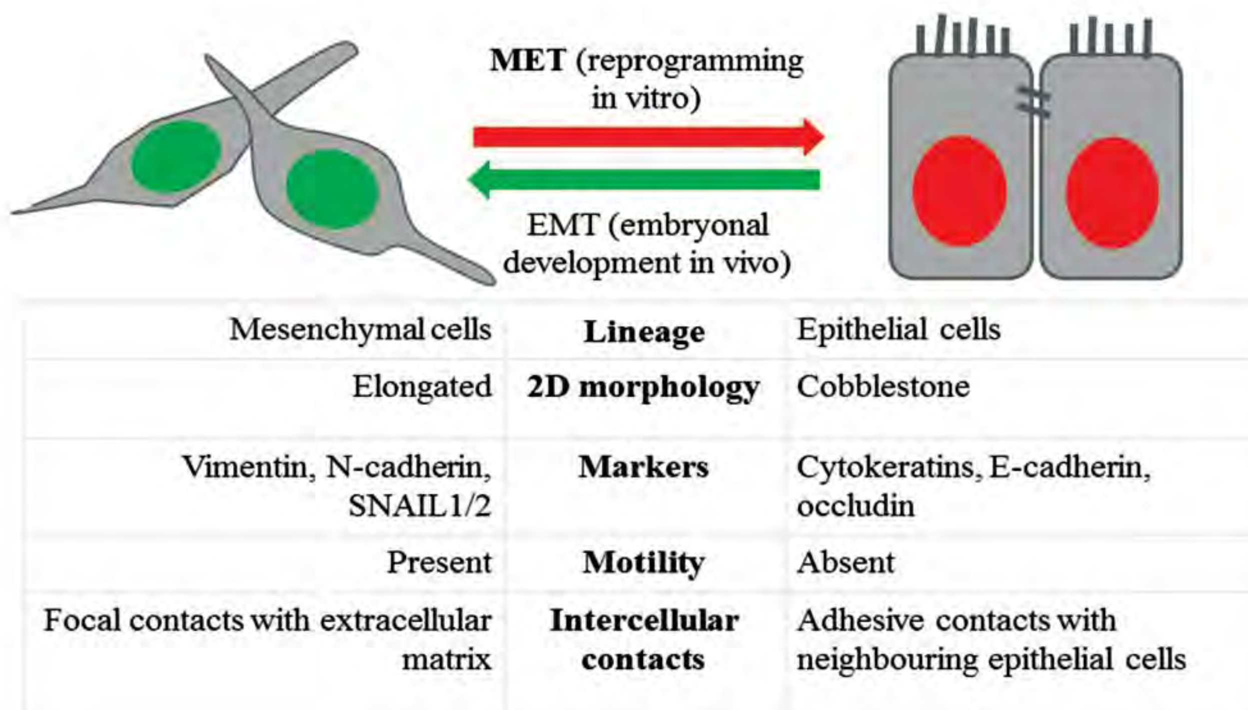
Metabolic changes determinant for the start of reprogramming, MET, the events of epithelialization with heavy loss of repressive histone modifications H3K27me3 and opening chromatin should be distinguished from the actual pluripotency induction. Genes responsible for epithelial phenotype, expressed in pluripotent cells, are not specific to the latter.

In addition, the abovementioned processes in spite of their high efficiency are reversible, such as in the case of TF removal the mesenchymal phenotype is restored. Therefore, a steady level of exogenous expression of TFs is a prerequisite for moving to the next phase of pluripotency induction [30].

Maturation of partially reprogrammed cells

It is worth noting that that not all works on TF-mediated pluripotency induction see this phase of molecular processes as a separate phase of the process of reprogramming. At the same time, given the transcriptome analysis data, many scientists isolate the maturation of partially reprogrammed cell (or later partial reprogramming) as a separate phase of pluripotency induction [13, 17, 30]. The basis for the separation of this phase is distinct specific gene expression profile observed after MET, which includes the expression of pluripotency genes FBXO15, SALL4, OCT4, NANOG and ESRRB and changes during the next phase of reprogramming [17, 30].

The decisive factor that stimulates the transition of partially reprogrammed human somatic cells to this phase is believed to



Mesenchymal cells	Lineage	Epithelial cells
Elongated	2D morphology	Cobblestone
Vimentin, N-cadherin, SNAIL1/2	Markers	Cytokeratins, E-cadherin, occludin
Present	Motility	Absent
Focal contacts with extracellular matrix	Intercellular contacts	Adhesive contacts with neighbouring epithelial cells

Fig. 1. **Mesenchymal-to-epithelial and epithelial-to-mesenchymal transitions, characteristics of mesenchymal and epithelial cells**

be LIN28. LIN28 is a marker of ESCs and enables the demethylation of promoters in abovementioned pluripotency genes, as well as the introduction of active histone modifications [30]. These very processes enable the further establishing pluripotency network, which happens in the next phase of reprogramming and will be described below.

The beginning of expression of FBXO15, SALL4, OCT4, after which the expression of NANOG and ESRRB is observed, is used by researchers to determine the transition of reprogrammed cell to the maturation phase, however, these markers are not indicative of the successful pluripotency induction and generation of functional iPSCs, namely the ability of self-maintenance when ectopic expression of reprogramming TFs is absent.

Scientists are pointing out the preparatory role of molecular epigenetic processes of this phase in ensuring the complete cell reprogramming through local chromatin remodeling and further derepression of pluripotency genes [9, 16, 30].

Deterministic phase of pluripotency network establishment and stabilization

Pluripotency network (Fig. 2) includes interacting TFs required to establish and maintain pluripotency [9]. Pluripotency network formation is a determinant factor of reprogramming success. Pluripotency network is built in a switch-like manner resulting in final transition of reprogrammed cells to pluripotency state and following the epigenetic alterations and activation of a few key genes [18, 31, 32].

The pluripotency network reestablishment is determined by endogenous SOX2 activation [16], requires removal of the ectopic TF expression and ensures stable functional pluripotency [18]. Epigenetic alterations that took place at the former reprogramming phases include accumulation of activating histone modifications cause formation of bivalent chromatin that is specific feature of pluripotency. In the pluripotent cells bivalent chromatin is responsible for specialization repression with sensitivity to multiple differentiation cues and specialization ability.

In PSCs bivalent chromatin is considered as consequent balance of two co-functioning protein complexes (repressive PcG and transcription activating TrxG). PcG and TrxG activities set repressive and activating histone modifications H3K27me3 and H3K4me3 on developmental genes promotors. In turn, the promotors can be repressed or activated depending on the specialization signals and enable cell fate choice (Fig. 3) [24, 33, 34].

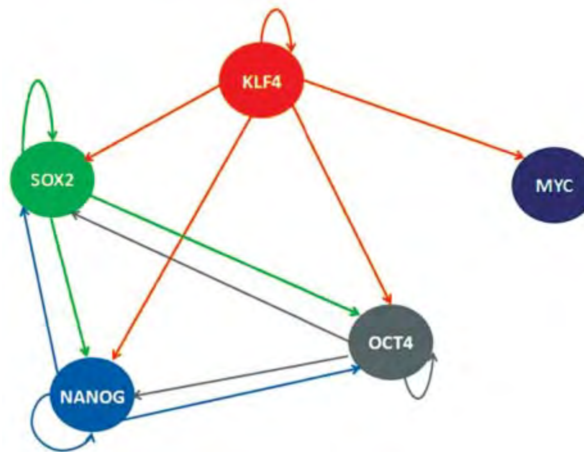


Fig. 2. **Interplay between master TFs in self-regulatory pluripotency network [32]**

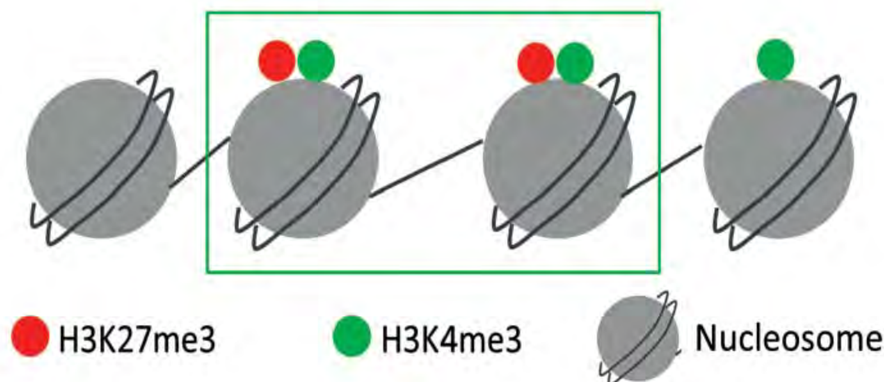


Fig. 3. **Bivalent chromatin with repressive H3K27me3 and activating H3K4me3**

Bivalent chromatin is a distinct feature of the functional pluripotency network at the later reprogramming phases. It arises as a result of local histone remodeling and emerging H3K4me3 that activate gene expression. It's important to note that the earlier phase shows the local histone remodeling at hypomethylated distal cis-regulatory sequences without expression of the relevant gene. Whereas accumulating chromatin alterations during the last two reprogramming phases allows for the establishment of bivalent chromatin. However, currently it's under discussion whether H3K4me3 and H3K27me3 marks are enough for the chromatin bivalency and functional pluripotency [18]. Therefore molecular algorithms of chromatin bivalency require further research in the field of somatic cell reprogramming.

Endogenous SOX2 activation and removal of ectopic TF expression are needed in order to establish pluripotency network and renew bivalent chromatin organization and epigenetic patterns of pluripotent cells [32, 35]. During pluripotency stabilization phase the successfully reprogrammed cells demonstrate abilities to self-renew and iPSC traits (e.g. expression of pluripotency genes and bivalent CpG-islands of development genes promoters, dynamic regulation of retrotransposons, etc.) [35–37]. As we mentioned above, activating histone modifications and KDM6A induced removal of repressive marks contribute to the derepression of pluripotency genes.

Thus stabilization phase efficiency is determined by following factors:

- silencing of the ectopic TF expression;
- expression of pluripotency genes;
- establishment of bivalent chromatin organization.

Bivalent chromatin required for the functional pluripotency is one of the key points reasoning the existing problems of cellular reprogramming process — modest efficiency and heterogeneity of iPSC lines [38].

Enhancing reprogramming efficiency and wider applications of transdifferentiated cells in disease modelling, pharmacology, cell therapy and regenerative medicine as well as other cell technology solutions require further investigation of molecular and epigenetic processes that take place in the phase described above. Identification of accurate algorithms in pluripotency network establishment, stabilization and in other pluripotency features allow for more effective iPSCs usage in multiple areas of biotechnology, medicine and agriculture [39–41].

So, within the reprogramming phases specified here the interactions network between transcription factors (especially OCT4, SOX2 and NANOG co-binding to the same target genes) is crucial in pluripotency induction and stabilization for somatic cells in mice and humans. OCT4 and SOX2 bind to the distal regions of proliferation and pluripotency genes that were inactivated during ontogenesis. C-MYC and KLF43 share these target regions to open the chromatin *de novo* or provide for active histone modifications (thus activating the gene expression patterns existing before cellular differentiation). Recent studies showed significant impact of OCT4 and C-MYC on cellular proliferation, metabolic shifts and MET during early reprogramming. Subsequent silencing of ectopic expression of reprogramming TF and endogenous SOX2 activation ensure the pluripotency network to set up with self-regulation mechanism due to self-maintaining expression of OCT4, SOX2 and NANOG. Finally, during the phase of pluripotency stabilization downregulated KLF4 expression being close to ESC levels contributes to reactivating DNA methylation regulators and completing the successful reprogramming of a somatic terminally differentiated cell into a pluripotent one.

One of the key barriers for iPSC applications is the multi-stage nature of somatic cells reprogramming that features both stochastic early phases and deterministic establishment of pluripotent regulatory network. Despite thousands of scientific studies, various reprogramming protocols restrict effective research analysis and identification of molecular reprogramming mechanisms. This variety also hinders scientists in their efforts to find the reprogramming efficiency drivers. In order to specify accurate reprogramming algorithms and develop more effective protocols of patient specific reprogrammed cells cultivation, the future researches require focus on the phase transition switchers.

REFERENCES

1. *Karpov O. V., Demydov S. V., Kyryachenko S. S.* Cellular and gene engineering. *Kyiv: Phytosociocenter*. 2010, 208 p. (In Ukrainian).
2. *Wu J., Yamauchi T., Izpisua Belmonte J. C.* An overview of mammalian pluripotency. *Development*. 2016, 143 (10), P. 1644–1648.
3. *Condic M. L.* Totipotency: What It Is and What It Is Not. *Stem Cells Dev*. 2014, V. 23 (8), 796–812.
4. *Gurdon J. B.* The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. *J. Embryol. Exp. Morphol.* 1962, 10, P. 622–640.
5. *Blau H. M.* Sir John Gurdon: father of nuclear reprogramming. *Differentiation*. 2014, 88 (1), 10–12. doi: 10.1016/j.diff.2014.05.002.
6. *Evans M. J., Kaufman M. H.* Establishment in culture of pluripotential cells from mouse embryos. *Nature*. 1981, 292, P. 154–156.
7. *Martin G. R.* Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc. Natl. Acad. Sci. USA*. 1981, V. 78, P. 7634–7638.
8. *Thomson J. A., Itskovitz-Eldor J., Shapiro S. S., Waknitz M. A., Swiergiel J. J., Marshall V. S., Jones J. M.* Embryonic stem cell lines derived from human blastocysts. *Science*. 1998, V. 282, P. 1145–1147.
9. *Kazutoshi Takahashi, Shinya Yamanaka*. A decade of transcription factor-mediated reprogramming to pluripotency. *Nat. Rev. Mol. Cell Biol*. 2016, 17 (3), 183–193. doi: 10.1038/nrm.2016.8.
10. *Takahashi K., Yamanaka S.* Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006, V. 126, P. 663–676. doi:10.1016/j.cell.2006.07.024.
11. *Yamanaka S.* Pluripotency and nuclear reprogramming. *Philos. Trans. Soc. Lond. B Biol. Sci*. 2008, V. 363, P. 2079–2087.
12. *Tian Z., Guo F., Biswas S., Deng W.* Rationale and Methodology of Reprogramming for Generation of Induced Pluripotent Stem Cells and Induced Neural Progenitor Cells. *Int. J. Mol. Sci*. 2016, V. 17, P. E594. doi:10.3390/ijms17040594.
13. *Samavarchi-Tehrani P., Golipour A., David L., Sung H., Beyer T. A., Datti A., Woltjen K., Nagy A., Wrana J. L.* Functional Genomics Reveals a BMP-Driven Mesenchymal-

to-Epithelial Transition in the Initiation of Somatic Cell Reprogramming. *Cell Stem. Cell*. 2010, V. 7, P. 64–77. doi: http://dx.doi.org/10.1016/j.stem.2010.04.01.
14. *Malik N., Rao M. S.* A review of the methods for human iPSC derivation. *Meth. Mol. Biol. Clifton. NJ*, 2013, V. 997, P. 23–33.
15. *Yamanaka S.* Elite and stochastic models for induced pluripotent stem cell generation. *Nature*. 2009, V. 460, P. 49–52.
16. *Kulcenty K., Wróblewska J., Mazurek S., Liszewska E., Jaworski J.* Molecular mechanisms of induced pluripotency. *Contemp. Oncol. (Pozn.)*. 2015, 19 (1A), A22–A29. doi: 10.5114/wo.2014.47134.
17. *David L., Polo J. M.* Phases of reprogramming. *Stem Cell Res*. 2014, V. 12, P. 754–761. doi: dx.doi.org/10.1016/ j.scr.2014.03.007.
18. *Smith Z. D., Sindhu C., Meissner A.* Molecular features of cellular reprogramming and development. *Mol. Cell Biol*. 2016, V. 17, P. 139–154. doi:10.1038/nrm.2016.6.
19. *Wang T., Warren S. T., Jin P.* Toward pluripotency by reprogramming: mechanisms and application. *Protein Cell*. 2013, 4 (11), 820–832. doi:10.1007/s13238-013-3074-1.
20. *Friedli M., Turelli P., Kapopoulou A., Rauwel B., Castro-Diaz N., Rowe H M., Ecco G., Unzu C., Planet E., Lombardo A., Mangeat B., Wildhaber B. E., Naldini L., Trono D.* Loss of transcriptional control over endogenous retroelements during reprogramming to pluripotency. *Cold Spring Harbor Lab. Press. Genome Res*. V. 24, 1251–1259.
21. *Maherali N., Ahfeldt T., Rigamonti A., Utikal J., Cowan C., Hochedlinger K.* A high-efficiency system for the generation and study of human induced pluripotent stem cells. *Cell Stem Cell*. 2008, 11 (3), 340–345. doi: 10.1016/j.stem.2008.08.003.
22. *Soufi A., Zaret K. S.* Understanding impediments to cellular conversion to pluripotency by assessing the earliest events in ectopic transcription factor binding to the genome. *Cell Cycle*. 2013, 12 (10), P. 1487–1491. doi: 10.4161/cc.24663.
23. *Chen T., Dent S. Y. R.* Chromatin modifiers and remodellers: regulators of cellular differentiation. *Nat. Rev. Genets*. 2014, V. 15, P. 93–106. doi:10.1038/nrg3607.
24. *Delgado-Olguín P.* Chromatin structure of pluripotent stem cells and induced pluripotent stem cells. *Briefings Funct. Genom*. 2011, 10 (1), 37–49.
25. *AlFatah M. A., Gafni O., Weinberger L., Zviran A., Ayyash M., Rais Y., Krupalnik V., Zerbib M., Amann-Zalcenstein D., Maza I., Geula S., Viukov S., Holtzman L., Pribluda A., Canaani E., Horn-Saban Sh., Amit I., Novershtern N., Hanna J. H.* The H3K27 demethylase Utx regulates somatic and germ cell epigenetic reprogramming. *Nature*. 2012, 488 (7411), 409–413.
26. *Bergsmedh A., Donohoe M. E., Hughes R., Hadjantonakis A.* Understanding the Molecular Circuitry of Cell Lineage Specification in the Early Mouse Embryo. *Genes*. 2011, 2 (3), 420–448. doi: 10.3390/ genes2030420
27. *Goyal A., Chavez S. L., Reijo Pera R. A.* Generation of human induced pluripotent stem cells using epigenetic regulators reveals a germ cell-like identity in partially reprogrammed colonies. *PLoS One*. 2013, 8 (12), e82838. doi: 10.1371/journal.pone.0082838.
28. *Megyola C. M., Gao Y., Teixeira A. M., Cheng J., Heydari K., Cheng E., Nottoli T., Krause D. S., Lu J., Guo S.* Dynamic migration and cell-cell interactions of early reprogramming revealed by high resolution time-lapse imaging. *Stem Cells*. 2013, 31 (5), 895–905. doi: 10.1002/stem.1323.
29. *Kida Y. S., Kawamura T., Wei Z., Sogo T., Jacinto S., Shigeno A., Kushige H., Yoshihara E., Liddle Ch., Ecker J. R., Yu R. T., Atkins A. R., Downes M., Evans R. M.* ERRs Mediate a Metabolic Switch Required for Somatic Cell Reprogramming to Pluripotency. *Cell Stem Cell*. 2015, V. 16, 547–555.
30. *Hawkins K., Joy S., McKay T.* Cell signalling pathways underlying induced pluripotent stem cell reprogramming. *World J. Stem Cells*. 2014, 6 (5), P. 620–628. doi: http://dx.doi.org/10.4252/ wjsc.v6.i5.620.
31. *Ye X., Weinberg R. A.* Epithelial–Mesenchymal Plasticity: A Central Regulator of Cancer Progression. *Cell. Trends in Cell Biology*. 2015, 25 (11), 675–686. doi: http://dx.doi.org/10.1016/j.tcb.2015.07.012.
32. *Johnson B. V., Shindo N., Rathjen P. D., Rathjen J., Keough R. A.* Understanding pluripotency — how embryonic stem cells keep their options open. *Mol. Human Reprod*. 2008, 14 (9), 513–520.
33. *Sha K., Boyer, L. A.* The chromatin signature of pluripotent cells, StemBook, ed. In: *The Stem Cell Research Community, StemBook*. 2009. doi: 10.3824/stembook.1.45.1.
34. *Sharma S., Kelly T. K., Jones P. A.* Epigenetics in cancer. *Carcinogenesis*. 2010, 31 (1), 27–36.
35. *Hussein S. M. I., Puri M. C., Tonge P. D., Benevento M., Corso A. J., Clancy J. L., Mosbergen R., Li M., Lee D.-S., Cloonan N., Wood D. L. A., Munoz J., Middleton R., Korn O., Patel H. R., White C. A., Shin J.-Y., Gauthier M. E., Lê Cao K.-A., Kim J.-Il, Mar J. C., Shakiba N., Ritchie W., Rasko J. E. J., Grimmond S. M., Zandstra P. W., Wells C. A., Preiss T., Seo J.-S., Heck A. J. R., Rogers I. M., Nagy A.* Genome-wide characterization of the routes to pluripotency. *Nature*. 2014, V. 516, P. 198–206.

36. *Ohnukia M., Tanabea K., Sutoua K., Teramotoa I., Sawamuraa Y., Naritaa M., Nakamuraa M., Tokunagaa Y., Nakamuraa M., Watanabea A., Yamanakaa S., Takahashi K.* Dynamic regulation of human endogenous retroviruses mediates factor-induced reprogramming and differentiation potential. *Proc. Natl. Acad. Sci. USA*. 2014, 111 (34), 12426–12431.
37. *Santoni F. A., Guerra J., Luban J.* HERV-H RNA is abundant in human embryonic stem cells and a precise marker for pluripotency. *Retrovirology*. 2012, 9 (111). doi: 10.1186/1742-4690-9-111.
38. *Budniatzky* **I.**, *Gepstein L.* Concise Review: Reprogramming Strategies for Cardiovascular Regenerative Medicine: From Induced Pluripotent Stem Cells to Direct Reprogramming. *Stem Cells Trans. Med*. 2014, 3 (4), 448–457.
39. *Hirschi K. K., Li S., Roy K.* Induced Pluripotent Stem Cells for Regenerative Medicine. *Annu. Rev. Biomed. Eng*. 2014. V. 16, P. 277–294. doi: 10.1146/annurev-bioeng-071813-105108.
40. *Kumar D., Talluri T. R., Anand T., Kues W. A.* Induced pluripotent stem cells: mechanisms, achievements and perspectives in farm animals. *World J. Stem Cells*. 2015. 7 (2), 315–328. doi: 10.4252/ wjsc.v7.i2.315.
41. *Baghbaderani B. A., Tian X., Neo B. H., Burkall A., Dimezzo T., Sierra G., Zeng X., Warren K., Kovarcik D. P., Fellner T., Rao M. S.* cGMP-Manufactured Human Induced Pluripotent Stem Cells Are Available for Pre-clinical and Clinical Applications. *Stem Cell Rep*. 2015, V. 5, P. 647–659.

МОЛЕКУЛЯРНІ МЕХАНІЗМИ ІНДУКЦІЇ ПЛЮРИПОТЕНТНОСТІ ТА ПЕРЕПРОГРАМУВАННЯ СОМАТИЧНИХ КЛІТИН

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Метою роботи було проаналізувати актуальні дослідження, присвячені молекулярним механізмам індукції плюрипотентності та основним етапам перепрограмування соматичних клітин. Особливу увагу приділено чинникам, що забезпечують перехід від одного етапу перепрограмування до іншого. Зроблено висновок про те, що однією з основних перешкод для здійснення IPSC є багатоетапний характер перепрограмування соматичних клітин, що передбачає наявність ранніх стохастичних фаз і детерміністське створення плюрипотентної регуляторної мережі. Незважаючи на численні наукові дослідження, різні протоколи перепрограмування обмежують ефективний дослідницький аналіз і виявлення молекулярних механізмів перепрограмування. Для пошуку точного алгоритму перепрограмування і розроблення більш ефективних протоколів перепрограмування культивованих клітин конкретного пацієнта у подальших дослідженнях слід зосередити увагу на індукторах фазових переходів окремих стадій перепрограмування.

Ключові слова: індукція плюрипотентності, перепрограмування, соматичні клітини, індуковані плюрипотентні стовбурові клітини.

МОЛЕКУЛЯРНЫЕ МЕХАНИЗМЫ ИНДУКЦИИ ПЛЮРИПОТЕНТНОСТИ И ПЕРЕПРОГРАММИРОВАНИЯ СОМАТИЧЕСКИХ КЛЕТОК

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Цель работы — проанализировать актуальные исследования, посвященные молекулярным механизмам индукции плюрипотентности, а также основным этапам перепрограммирования соматических клеток. Особое внимание уделено факторам, обеспечивающим переход от одного этапа перепрограммирования к другому. Сделан вывод о том, что одним из основных препятствий для осуществления IPSC является многоэтапный характер перепрограммирования соматических клеток, что предполагает наличие ранних стохастических фаз и детерминистское создание плюрипотентной регуляторной сети. Несмотря на множество научных исследований, различные протоколы перепрограммирования ограничивают эффективный исследовательский анализ и выявление молекулярных механизмов перепрограммирования. Для поиска точного алгоритма и разработки более эффективных протоколов перепрограммирования культивируемых клеток конкретного пациента в дальнейших исследованиях необходимо сосредоточить внимание на индукторах фазовых переходов отдельных стадий перепрограммирования.

Ключевые слова: индукция плюрипотентности, перепрограммирование, соматические клетки, индуцированные плюрипотентные стволовые клетки.