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REVIEW

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Cellular Reprogramming: Paradigm of Futuristic Medicine

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Abstract

In the past twenty years, the discourse of "Cellular Reprogramming" has gone from fundamental science to the science of " applied bioengineering", with workers working feverishly to recreate a variety of cell types. Once thought as irretrievably differentiated, mature cells are now seen to be "flexible entities" capable of switching or "flip-flopping" from one form to another with relatively simple manipulation. Although onset of induced pluripotent cells from the reprogrammed somatic cells by spatial and temporal expression of several transcription factors is a well-documented phenomenon in today's cellular differentiation understanding, the genetic basis of such is still poorly understood. Here in this review, we attempt to analyze the current advances of the rapidly growing field of cellular reprogramming and differentiation, along with putative directions of future research involving the same.

Keywords: *Differentiation, Pluripotency, Metaplasia, iPsc, cardiomyocytes*

Introduction

Cellular reprogramming is the conversion of one tissue-specific cell type to another, usually induced by specific and/ or several transcription factor dependent manners. Shen *et al* had observed that some of the rat pancreatic exocrine cells suddenly changed phenotype, and become unusually large and flattened [1]. After careful analysis he identified the reason for this switching of cellular shape. The cells, which belonged to an established line termed AR42j, were no longer pancreatic cells but surprisingly they underwent a "Cellular Flip-flop" to liver cells or hepatocytes. Under the influence of the synthetic hormone, dexamethazone, which had been added to the medium to enhance endocrine cell secretion, the cells changed their identity.

However this was not the first case reported were cells metamorphosed. Metaplasia, a relatively harmless process, was long reported by pathologists, in which cellular flip-flop occurred [2]. The AR42j underwent "Transdifferentiation", paved the way for the development of a convenient model to study cellular reprogramming, which enables the researchers to look at the immense possibilities of this cell line.

The ability to generate a plethora of cell types have immense potential and if effectively applied can open totally unchartered avenues for further biomedical research and novel therapeutic approaches [2]. It is now very much a reality to create cultures of human cells, such as neurons, hepatocytes or cardiomyocytes, that would be really difficult to procure from animal models and normal and diseased persons. Researcher can now derive skin or blood samples from patients suffering from dreaded diseases and apply those cells to derive disease-specific stem cell lines which can be reprogrammed to generate an immense variety cell types [2]. Its just a matter of time when we all will see that cell reprogramming technologies offering one day source of useful cells for therapeutic transplantation to treat diseases such as diabetes, Parkinson's to name a few.

The foremost objective for the researchers is to understand the cell signaling pathways that are triggered by cellular reprogramming in which temporal and spatial expression of genes in cells results in the generation of a different morphology are indeed "permanent" or "reversible". If the

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process is reversible, then probably it will be of little use in transplantation studies [2].

A Bird's eye view of Cellular Reprogramming

Any cell type normally contains relatively large quantities of few proteins like muscle expresses actin and myosin, while neurons have synapsin and neurofilaments. Some differentiated cells can persist for a lifetime, such as neurons and muscle cells, while others exhibit a finite lifetime, in which progenitor cells under the influence of cytokines and other growth factors continuously replace the dying cells [2]. For many years it was established that mature cell types are stable entities, which could not be reprogrammed into different varieties of cells. Recently this notion have been changed, since over-expression of certain transcription factors (TFs) has been successful in reprogramming of cells, that is a dramatic transformations occurred, which was thought to previously as an impossibility. An appropriate example would be the regression of adult fibroblasts to stem cell like states, and the "Direct Reprogramming" of the same towards the skin cells and neurons [2]. It is now apparently clear cells switch from one form to another during normal course of development. For instance the epithelial lining of the esophagus is formed as a columnar epithelium like the remaining part of the gut. Surprisingly later, it transforms into a stratified squamous epithelial type resembling the cells of the skin [3].

Pancreatic endocrine cells similarly are also observed in the bile ducts of the liver. This change takes place late in embryogenesis, involving a "cellular flip-flop" from a ductal to an endocrine variety [4]. In Drosophila "Transdetermination" is characterized by cellular metamorphosis. The Fly imaginal discs of the developing larvae are sometimes "Recoded" during regeneration, so that an incorrect body part that is a leg or alternatively a wing develops due to aberrant expression of TFs [5]. "Serial Metamorphosis" involves replacement of an appendage by one normally found elsewhere in the body, is observed both in crustaceans and insects [5]. In a similar way cellular switching or flip-flopping is rarely if ever seen in adult vertebrates, like newts and salamanders, where it is seen that the "lens" of the eye can be regenerated after removal of it. If the lens is experimentally removed, the cells of the dorsal iris proliferate, undergoes depigmentation, but ultimately re-differentiating to form a new lens [6]. Here the cell types are radically different: whereas cells of the iris are pigmented epithelial cells, similar to those of retinoid cells, the lens in contrast is composed of modified keratinocytes, containing extremely high concentration of crystallin proteins, which imparts its characteristic transparency. Surely this lens system suggests that there is a connection between wounding, which is a regenerative process, and cellular flip-flopping [6].

In human tissues it is not improbable to observe small bits of "non-resident" tissues as seen when we find deposits of minute bones in soft connective tissues, or even patches of skin like epithelial cells in an epithelium, which is essentially glandular [7]. In fact gastro-intestinal layer and female reproductive tissue can become "Metaplastic" [7]. It should be noted that these locations are always subjected to external and internal stressors like chronic trauma, infection with microbionts or hormonal stimulation, leading to regeneration of cells or metaplasia. Surprisingly the misplaced aberrant tissue types is often the same as that derived from cells which are in reality neighbors in the developing embryo, which give rise to the host tissue of the metaplasia. Again it is observed that patches of intestinal tissue occasionally form in the mucosal layer of the stomach. It is known that that intestine and stomach develops from neighboring zones in the ectoderm of the early embryo [7].

In the same category, large intestine type of tissue can form in the urinary bladder, a condition termed as "Cystititis glandularis". It is certainly a remarkable finding that bladder is quite separate from the gut in adults, but it is derived from neighboring endoderm in the embryo. Most of the "metaplasias" are in fact harmless, but some are associated with a moderate risk of developing into neoplasms or cancers [7]. For instance patches of squamous tissues are generated in the columnar epithelial lining of the bronchii of the lungs in smokers, and these have a central tendency to sprout into a type of lung cancer. Again adenocarcinoma of the esophagus usually forms in zones of Barret's metaplasia, a condition in which the normally squamous epithelium of the lower part of the esophagus suffers "switching" to columnar epithelial type exhibiting a differentiation pattern typical of the intestinal niche. So as per the proverb" In Rome behave like Romans" or "Be a roman in Rome" is certainly applicable here in the human body as well.

Traversing the path of Cellular Reprogramming

Early experimental observations aimed at altering the fate of cells relied on grafting of patches of growing cells from one region of the embryo to another, where a plethora of signaling factors instigated their acquired behavior in the new niche [7]. This type of experiment is radically different from modern reprogramming which involves the introduction of TFs into cells [8]. These phenomenal studies led to the discovery of several key-signaling pathways in the process of development, as for example, the "Wnt" and "Hedgehog" pathways, in the late 1980s and early 90s. Then came the year 2006, certainly a landmark year, which saw the development of the "Science of Reprogramming". Shinya Yamanaka and his colleagues of Kyoto University, announced a revolutionary finding. - "A novel strategy for reprogramming cells" [9]. By engineering the over-expression of a mixture of pluripotency associated TFs in mouse fibroblasts, Yamanaka's group were successful in generating the world's first Pluripotent Stem Cells (iPSCs) (Figure 1) [9].

This remarkable achievement initiated other laboratories to produce iPSCs by a variety of transcription factors/genes expressing a range of TFs in cells of model animal systems or human iPSCs are in reality very much similar to ESCs or

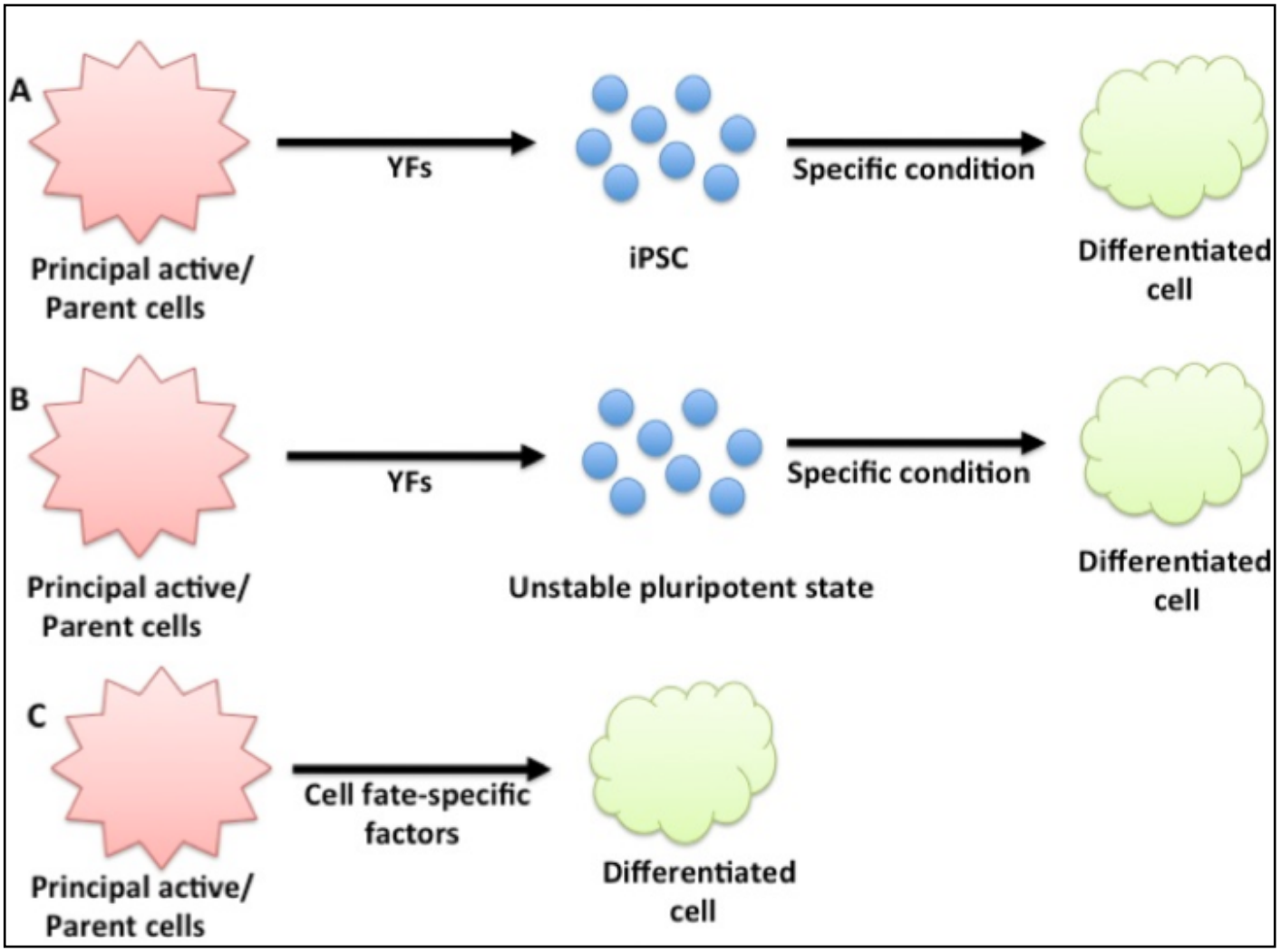

Figure 1: Types of cellular reprogramming and their fate-specific differentiation under precise environmental condition. **(A)** Yamanaka factors (YFs) induced reprogramming of induced pluripotent cell (iPSC) and their specific cellular differentiation. **(B)** Differentiation of specific cellular types from YFs induced unstable pluripotent cells. **(C)** Direct lineage conversion of principal active/ parent cells by cell-fate specific factors. (Diagram is taken, conceptualized and modified from Lujan and Wernig 2013 [16])

Embryonic Stem Cells. First and foremost they can grow endlessly that is they are pluripotent. When iPSCs are exposed to appropriate media, they are instigated to differentiate into a wide variety of cell types that is both permanent and discrete at the same time. Mouse iPSCs will naturally find settlement in the mouse embryo; capable of contributing in the formation of different types of tissues and even germ line can be generated. In vitro human iPSCs have been re-differentiated to nearly all cell types, viz, neurons, hepatocytes and cardiomyocytes [9]. These iPSCs are seen as valuable model systems for doing research in both human development and diseases, as a convenient and reproducible system for obtaining a variety of cell types for drug testing. And last but not the least iPSCs finds permanent application in therapeutic transplantation.

Workers all around the world have refined Yamanka's original technique to increase the efficiency of transformation by opening chromatin to increase the accessibility of target genes for various TFs. The number of cells that can be reprogrammed increased dramatically from 1 in 100,000 to 1 in 1000 [5]. Even 100% transformation efficiency was reported from some laboratories. Again iPSCs can be generated from cell types, which are different from fibroblasts. Specifically "Lymphocytes" is isolated from blood, which can serve as the starting material for cell differentiation, making it very much convenient and easier to form iPSCs from individual patients. Stem cell biologists have also tried to avoid integrating vectors, such as retroviruses and lentiviruses, which on their part help in the incorporation of experimental TF genes, in the genome of the host. This is done to negate the possibility of introducing any harmful mutations, which can give rise, at least potentially to carcinomas. The non-integrating vehicles for the introduction of of TF genes can be either excisable, or also RNA molecules which are certainly safer than integrating vectors. Various small molecules can be ideal substitutes in place of original reprogramming TFs and this is proven by recent studies, which demonstrated, how "Pluripotency" per se could be induced in differentiated adult mouse cells with a rather simple enough

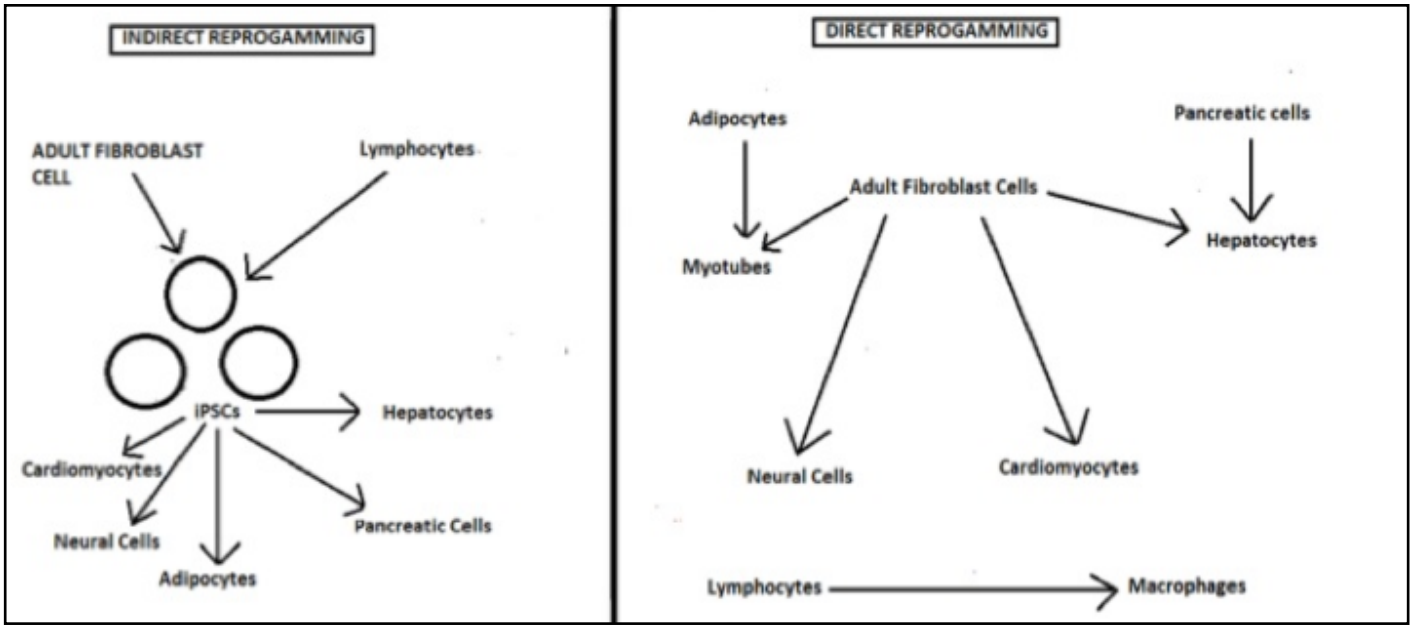

Figure 2: Somersaulting between cells: Contrasting features between direct and indirect programming. Over expression of just a handful of transcription factors (TFs), especially those which are involved in embryonic development are enough to instigate these dramatic flip-flopping in induced pluripotent stem cells (iPSCs). While the combination of these TFs is conventional in generating iPSCs in a differentiation fate dependent manner, related regulatory small molecules including other TFs, miRNAs, and non-related genes such as lineage factors can functionally replace each of the factors, essentially mimicking the regulatory functions of iPSC flip-flopping TFs.

external stressor like lowering of pH of the medium in which the cells are growing.

However it should be absolutely borne in mind, that despite their useful qualifications, and also their well-known similarities with ESCs, iPSC cell line would at least carry some "Memory" from the parental cell/primordial cell from which it is derived [7]. These are encoded or carried forward in the progeny cells as specific DNA methylations. The latter are epigenetic markers, which may bias the cells to wards differentiation into parental types. In sharp contrast ESCs do not carry any "pas baggage" or biases as they are derived from cells that are "yet-to-be differentiated into "ICM" or Inner Cell Mass of the early embryo. Another significant facet of research in reprogramming is the "direct conversion of one cell type to another by means of "over-expression of specific transcription factors". The primary instance of cells changing types without pluripotent stage was the seminal discovery of MyoD by Harold Weintraub in 1989. MyoD by itself or independently can reprogram a variety of tissue culture cell lines into multi-nucleated muscle fibers termed "myotubes" [10]. Researchers have now achieved innumerable direct con versions after Weintraub's discovery. This includes the switching of pancreatic exocrine cells into hepatocytes or other exocrine cells into hepatocytes or other endocrine cells; B-lymphocytes into macrophages; and fibroblasts into neurons, cardiomyocytes and hepatocytes. These set of four transcription factors serve primarily in the activation of certain genes and very importantly it can act as "silencers" and shift the cell to a permanent state of new gene expression [10].

Certainly the reprogramming and hence transformation per se appeared to irretrievably permanent, characterized by absolutely no reversion to a primordial cellular phenotype, which was followed by normal development, as has been shown by direct reprogramming or conversion induced prospective generation of pancreatic beta cells. The transplantation of islet cells is unquestionably a very efficient and an instance of successful therapy. Unfortunately there was a shortage of donors, which is quite natural. Beta like cells could certainly fill this gap, and if generated from Mesenchymal Cells (MSCs) of the individual patient, certainly could avoid immune rejection of the transplanted cells [10].

Traversing A New Territory

An important of "Cellular Reprogramming" is how the incorporated transcription factors find their gene targets in closed chromatin [11]. As we know much of the DNA is wrapped around "Nucleosomes" and covered by Histone proteins. The gene expression can further be repressed by higher order chromatin structures like loops and domains. Genome wide localization studies have reflected that almost all transcription factor-binding sites remains unoccupied, suggesting that most nuclear DNA is inaccessible to TFs. Interestingly some transcription factors could recognize their target genes even in closed chromatin. Interestingly some transcription factors could even recognize their target genes in closed chromatin [11]. MyoD was the major discovery and this pioneer transcription is responsible for direct reprogramming in intact/closed chromatin. Moreover if MyoD's transcription activation domain in the gene is added to Oct4 gene sequence, then the Oct4 protein formed a transcription factor in its own

right, influences and maintains pluripotency and in fact this communion is much more potent in generating more iPSCs in minimal time than wild type Oct4 alone [12].

Here the mixture used for direct reprogramming usually requires at least one "pioneer" factor. The experimental excision *ofMbd3* the gene for a component of a major chromatin repressor complex could emphatically augment the efficiency of the generation of iPSCs [12]. As a result of these pioneering studies it is now feasible to derive an iPSC line from virtually any patient sample. The essential similarity of the course of development of the two cell types can also affect the process and progress of cellular reprogramming. Fundamentally the Hierarchical nature of normal development might suggest that the chromatin states of similarly related tissue types (like the different types of epithelium) are more similar than those distantly located tissues [13]. If this is taken to be the gold standard, the TFs that are used to instigate the expression of cell-specific genes, should certainly have a much easier access to their target genes; thus paving the way for efficient cellular reprogramming.

This hypothesis was subsequently supported by many intuitive experiments. Primarily pancreatic like cells were generated from liver cells and the cardiomyocytes were reprogrammed from cardiac fibroblasts. On a similar note, several recent advancements occurred in the facet of direct reprogramming of fibroblasts, which suggests that it is, very much possible to overcome any hurdle by high doses of high quality TFs [13] (Figure 2). At this juncture it is important to realize that "cellular reprogramming" does not just demonstrate up regulation of various genes by the incorporated transcription factors. Rather the TFs used for such processes normally have a number of differentiated gene products among their essential gene targets. Fortunately the genes are only expressed as long as the introduced TFs are present. Once these are degraded there would be a reversal of phenotype [13]. So it is to note that "Real Reprogramming" per se certainly involves an irreversible switch of phenotype that persists despite the slow degradation of TFs that originally was instrumental for the transformation [14].

In the instance of iPSCs there is not even a ray of doubt that the cells have undergone an irreversible change since the cells become essentially stem cells, dividing endlessly with stability. With direct reprogramming however it may be more worthwhile to test whether gene expression pattern is permanent [14].

Perusal of non-integrating vectors such as Adenoviruses or Adeno associated Viruses (ANV) to introduce the transcription factors ultimately leads to loss of viral DNA, but the bottom line is that this process takes a long time. Inducible systems as for example those systems that can be regulated by the incorporation of certain drugs, can help in the control of the time taken in which TFs are expressed. In this instance "What will happen if there is withdrawal of the drug?" It is seen apparently that some residual gene expression remains, even after removal of the drug [14]. Using gene delivery viruses it has been observed that TFs when administered in intact animals can be much less of a daunting aspect than that of in vitro incorporation. This is entirely surprising, as one might opine that the complex organ environment, with various cellular repertoires, and altogether an entirely different niche and a variety of external stimuli, would certainly have a tendency to stabilize a cell.

Again it is certainly feasible to induce iPSCs in mice, where tumorigenesis occurs known specifically as "teratomas". The latter are made up of pluripotent stem cells as well as many other cell types [14]. Again it is possible to transform cardiac fibroblasts to cardiomyocytes in live adult mice [15] and to partially transform pancreatic exocrine cells to endocrine cells [15].

It is however notable that naturally occurring cell type transformation or flip-flopping are rare in vivo, but provoking these cells offers yet another avenue therapeutic research.

Perspectives for the Future

A bird's eye view of such an important area of scientific accomplishment, does not do justice to the immense possibilities of different types of stem cells be it ESCs /MSCs/ iPSCs. Nowadays huge potential is seen in Chord Blood Cells, amniocytes 1 and 2, cells of the gum tissue surrounding the teeth, etc. If these can be used and channelized in futuristic medicine, then absolutely new and unchartered territories can be opened up for further development.

Conflicts of Interests

The authors do not have any direct financial relation that may lead to a conflict of interests for any of the authors mentioned herein, and there is no conflict of interests regarding the publication of this article.

References

1. Shen CN, Slack JMW, and Tosh D: **Molecular basis of transdifferentiation of pancreas to liver.** Nat Cell Biol 2000, **2**: 89-87.
2. Slack JMW: **Homoeotic tansformation in man; implications for the mechanism of embryonic development and for the organization of epithelia.** J Theor Bio. 1985, **114**: 463-90.
3. Yu WY, Slack JM, and Tosh D: **Conversion of columnar to stratified squamous epithelium in the developing mouse esophagus.** Dev. Biol 2005, **284**:157-70.
4. Dutton JR, Chillingworth NL, Ebarhard D, Brannon CR, Hornsay MA, Tosh D, Slack JM: **Beta cell occurs naturally in extrahepatic bile ducts of mice.** J.Cell.Sci 2007, **120**:239-45.
5. Maves L, Schubiger G: **Trandetermination in Drosophila imaginal discs: a model for understanding pluripotency and selector gene maintainence.** Curr Opin Genet Dev 2003, **13**: 472-79.
6. Tsonis PA, Madhavan M, Tancous EE, DelRio-Tsonis KP: **A newt's eye view of lens regeneration.** Int. J Dev Biol 2004,

46:975-80.

7. Willis RA: The Borderland of Embryology and Pathology, Butterworth; 1962.
8. Slack JMW, Essential Developmental Biology 3rd ed., Wiley-Blackwell; 2012.
9. Takahasi K, Yamanaka S: **Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors.** Cell 2006, **126**:663-76.
10. Weintraub H, Tapscott SJ. Davis RL. Thayer MJ, Adam MA, Lassar AB, Miller AD: **Activation of muscle-specific genes in pigment, nerve, fat, liver, and fibroblast cell lines by forced expression of MyoD.** PNAS 1989, **86**: 5434-38.
11. Zaret KS, Carroll JS: **Pioneer transcription factors: establishing competence for gene expression.** Genes Dev 2011, **25**:2227-41.
12. Hirai H, Tani T, Katoku-Kikyo N, Kellner S, Karian P, Firpo M, Kikyo N: **Radical acceleration of nuclear reprogramming by chromatin remodeling with the transactivation domain of MyoD.** Stem Cells 2011, **29**:1349-61.
13. Rais Y, Zviran A, Geula S, Gafni O, Chomsky E, Viukov S, Mansour AA, Caspi I, Krupalnik V, Zerbib M, Maza I, Mor N, Baran D, Weinberger L, Jaltin DA, Lara Astiaso D, Blecher-Gonen R, Shipony Z Mukamel Z, Hagai T Gilad S, Amann Zalcenstein D, Tanay A, Amit I, Novershtern N, Hanna JH: **Deterministic direct reprogramming of somatic cells to pluripotency.** Nature 2013, **502**:340-45.
14. Qian L, Huang Y, Spencer CI, Foley A, Vedantham V, Liu L, Conway SJ, Fu JD, Srivastava D: **In vivo reprogramming of murine cardiac fibroblasts into induced cardiomyocytes.** Nature 2012, **485**:593-598.
15. Zhou Q, Brown J, Kanarek A, Rajagopal J, Melton DA: **In vivo reprogramming of adult pancreatic exocrine cells to beta-cells.** Nature 2008, **455**:627-32.
16. Lujan E., and Wernig M: **An indirect approach to generating specific human cell types.** Nature Methods 2013, **10**: 44-45; doi: 10.1038/nmeth.2325.

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