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Perspectives on Cell Reprogramming with RNA

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Abstract

Recent advances in cell reprogramming have permitted the development of different stem cell lines and specific differentiated cell types using distinct technologies. Cell reprogramming is largely mediated by DNA and RNA. In this review, we explore the RNA mediated cell reprogramming to induce specific target cell generation including stem cells, brain cells and cardiac cells. The ability of RNA populations to produce direct cell to cell phenotypic conversion is called Transcriptome Induced Phenotype Remodeling (TIPeR). The theory and utility of RNA use for cellular reprogramming is explored in this review.

Keywords

RNA-mediated; transdifferentiation; TIPeR; transcriptome transfer; RNA; cellular reprogramming

Cellular reprogramming

The historical view of cellular development states that cells require specific cues to proliferate and differentiate, and then require guidance to form tissues and further organize into complete organs. This is a unidirectional process culminating in cells that are terminally differentiated in a manner based on their lineage. According to this long-standing theory, this is caused by an irreversible loss in expression of functional genes within that particular cell as well as the activation of selective genes.

All information in cells and tissue is essentially derived from the master blueprint of DNA. This suggests that, transferring the DNA from one cell to another should transfer all information about the donor to the recipient. To test this theory, many laboratories have used the technique of somatic nuclear transfer (SNT), pioneered by *Xenopus* somatic nuclei transplants to make tadpole clones [1]. Many years later, this technically difficult procedure was successful in mammalian cells, most famously producing the cloned sheep, Dolly [2, 3]. This and other such successes provided hope that these techniques would result in production of cells that will have regenerative medicinal uses such as the production of specific organs or cells for therapeutic use in long-term or terminal diseases [4].

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In the early 1960's, a series of experiments were performed where in mice experienced whole-body radiation followed by transplantation of bone marrow from normal mice. The bone marrow cells repopulated the irradiated mice with normal circulating cells. These experiments yielded the first hints of the existence of undifferentiated but proliferative cell types, which were later defined as stem cells [5]. This was an exciting development because in addition to a potential for cloning an entire animal, stem cells offered immediate potential for clinical applications such as cell therapy without the need for a complicated processes such as SNT. Further, the pluripotency of these cells implied greater cell-type availability for various therapeutic uses. In addition to embryonic stem cells, the discovery of the presence of adult stem cells has also generated great interest in anticipation of clinical applications including transplantation [6].

Most recently based on earlier genomic research in stem cells, a subset of transcription factors was used to generate induced pluripotent cells (iPS) [7]. The selection of highly expressed transcription factors in ES cell lines and frequently upregulated genes were utilized to induce stem cells. This approach has rapidly stimulated research in reprogramming somatic cells into pluripotent stem cells. Subsequent research has focused on combining different transcription factors for more efficient stem cell generation and different transfection strategies [8–12]. These initial iPS studies utilized promoter driven cDNA constructs as the transferred agents to induce phenotypic change upon expression in fibroblasts. This DNA transcription factor-mediated methodology has also been used to transdifferentiate (directly convert without going through the stem cell intermediate) fibroblasts to iCardiomyocyte (induced Cardiomyocyte-like cell) and also pancreatic exocrine cells into β -cell-like cells *in vivo* [13, 14].

Simultaneously a distinct but novel approach was developed. Here a host cells's phenotype was directly converted by transplanted mRNA from a donor cell. The host cell then undergoes a phenotypic conversion and stably expresses the donor cell phenotype [15]. This method has been used to convert post-mitotic neurons have been converted to tAstrocytes while fibroblasts as well as astrocytes have been converted into tCardiomyocytes [16].

There are three fundamental differences between these approaches (Figure 1). (i) In nuclear transfer, the innate programming capability of the totipotent cell is used to drive transformation. (ii) In iPS cells, transcription factors are used to drive quiescent DNA transcription. (iii) In TIPeR, the RNA complement present in a functional cell is introduced to transform the cell state. In this review, we will focus on recent research in inducible stem cell generation and direct cell differentiation by phenotype remodeling. A detailed discussion of the methodological differences between these techniques is beyond the scope of this review, and we will instead discuss the process of cell differentiation through reprogramming and remodeling, comparing the concepts and the hopes for such technologies[17].

Nuclear transfer

Despite its low efficiency and unavoidable ethical issues, nuclear transfer is the easiest available method to produce totipotent cells that will most closely mimic the natural cell lineage development process. Totipotency is only present and maintained through very early stages of development following fertilization; cells generated from ES lines are by definition pluripotent because they are derived from cells in later stages of development. This method has been used recently to generate pluripotent embryonic stem cell lines from mammals including mice and monkeys [18, 19]. So far, a cell line from human origin has not yet been generated using this method, the prospect of which faces ethical roadblocks [20]. Currently available totipotent cells obtained from animal models, combined with genomics and

proteomics analytical tools, can be used to characterize the gene set that is necessary for self-renewability and the maintenance of pluripotency, including the transcription factors required at specific developmental stages.

DNA-mediated introduction of transcription factors to induce phenotypic conversion

Based on genomics level transcriptome analysis of stem cell gene activation, inducible stem cells have been produced using four transcription factors. The method has generated new possibilities for understanding how to reprogram somatic cells to dedifferentiate. This is akin to the development of a continuous cell line upon induction of cancer by an oncogene. Using selected transcription factors, maintained at high abundance in pluripotent stem cells, terminally differentiated somatic fibroblasts were induced to become pluripotent stem cells through reprogramming. These cells have a phenotypic resemblance to embryonic stem cells and are capable of differentiation into neurons and cardiomyocytes [21, 22]. Hence, somatic cells are capable of dedifferentiation into self-renewable stem cells.

DNA mediated iPS technology provides an alternative to nuclear transfer that is easily and immediately applicable to the pharmaceutical industry for efficient drug design and for testing efficacy within a uniform population of cells sharing the same status through tight control of cell lineage. However, some aspects of the generation of these cells should still be taken into consideration when contemplating their use. Retroviral insertion of transcription factors into the genome occurs randomly, allowing no control over the insertion site and potential for the development of mutagenesis. Digital quantification of mutations has revealed that multiple mutations are present in many different existing iPS cell lines that are currently in use [23]. In order to address this issue, alternative methods such as transduction with adenovirus vectors or transfection using plasmid vectors has been used to generate iPS cells but at a significant loss of efficiency compared to retroviral transduction[24–26]. This suggests that the overexpression of transcription factors may be necessary to gain highly efficient iPS cell generation. A cautionary aspect of viral mediated generation of iPS cells is that viral vector transduction without reprogramming transcription factor genes also produced iPS cells, which suggests that vector-induced insertional mutation caused somatic cell reprogramming [27].

For use in regenerative medicine, it is critical that cells introduced for treatment are stably differentiated and will not develop into undesired cell types [28, 29]. Many of the commonly employed iPS factors are enriched in pluripotent blastoma cells, for example, the well-known oncogenes Oct4 and Myc. This enrichment raises concerns that the over-expression of these factors may not result in generation of a long-term stable phenotype [30, 31]. An alternate approach used by the other groups, entails the generation of iPS cells by omitting oncogenes and using DNA-mediated introduction of other transcription factors [9–11]. Induction of epiblast stem cells using the DNA-driven iPS method with modified culture conditions [32, 33]. This finding demonstrates that a subpopulation of inhomogeneous cells may be generated using current methods for producing iPS cells, and may potentially present a future risk. The extent of this population has not yet been determined. The ability to use autologous somatic cells for induction of iPS and subsequent cell types from a patient, is expected to reduce issues related to immunohistocompatibility[34]. Unexpectedly, however, in a recent report highly expressed levels of Zg16 and Hormad1 were observed in transplanted differentiated cells, resulting in transplant immunorejection [35]. Therefore, prior to clinical application, further research and verification of long-term stability of differentiated cells from iPS intermediates should be undertaken.

Considerations with regard to cell phenotype

Recent progress in genomics and proteomics research reveals significant variation in protein and RNA abundances between individual cells that share a common phenotype [36]. Phenotypic marking is essential to isolate lineages after a differentiation protocol in order to raise target cells from stem cells. However, stem cell phenotypic identification may not be as straightforward as identifying cell types from a fixed sample. Cellular phenotype results from the orchestration of genomic and proteomic activities and is influenced by biochemistry and physiology. It is important to view these biological activities as dynamic regulators that act to maintain the cell's state within given phenotypic boundaries mRNA and protein quantities are likely to be present at set ratios in fully differentiated cells, but are likely to vary widely within the stem cell in response to signals from environmental or genetic cues. Therefore, the general upstream (unregulated transcription factor expression after transfection) genetic modification of cell fate regulators may increase the risk that a cell is directed to an unexpected lineage.

Ideally, identifying a precise gene network interaction map in conjunction with an accurate and precise method for inserting known amounts of transcription factors or other modulators of transcription, would make transcription based reprogramming of somatic cells to stem cells a good choice for clinical and therapeutic applications. Even though there are many methods to introduce transcription factor DNAs into to a cell, elimination of nonspecific gene activation following differentiation is difficult. DNA-mediated introduction of transcription factors requires a promoter, exogenous or endogenous, to drive expression of the transgene. Exogenous promoters are difficult to control even with the presumably regulatable promoters, usually resulting in aberrant over-expression of the transgene. This problem becomes more serious in therapeutic applications.

mRNA induced phenotype conversion

Phenotype generation through direct cell transdifferentiation is preferable to having to go through the intermediate pluripotent stem cell stage. Recent studies designed to assess the importance of expression profiles (relative RNA abundances) in cells, demonstrated that it is possible to convert cell phenotypes through transfer of mRNA populations. This method, called Transcriptome Induced Phenotype Remodeling (TIPeR) is used to directly transfer cell phenotypes without using vectors and eliminates the need to first de-differentiate followed by re-differentiation. This was first exemplified using cells from the central nervous system. Neurons and astrocytes are both derived from the same ectodermal tissue, but gain different function, physiology, and anatomy during development, and accordingly express different proteins. Using TIPeR, neurons were converted to astrocyte-like cells as determined by quantitation of multiple phenotypic measures. These results demonstrated the viability of the TIPeR approach in eliciting somatic cell to cell conversion. This approach has several advantages over DNA-mediated phenotypic conversions. In this method, using specific RNAs avoids activation of non-relevant genes and pathways driven by the difficult to control promoter-driven over-expression of transcription that may incorrectly activate networks of gene expression that are inappropriate in a specific desired cell type. Transcriptome transfer from donor cell to the host cell modifies the level of various mRNAs within the host cell mRNA population. This modification of the transcriptome is achieved by either repetition of the TIPeR process or increasing the amount of mRNAs added until a sufficient level is reached to drive phenotypic conversion to a stable epigenetic landscape (Figure 2) [36]. A major advantage of using mRNA to drive transformation is that its expression in the host cell is transient due to the cellular degradation of mRNA. Once the transfected mRNA has been translated it is degraded by natural cellular processes and will not generate unwanted long-term effects. In addition to this, the donor cellular mRNAs used

in some TiPeR methods also include all forms of the destination cell mRNA population including various alternatively spliced and edited forms. Therefore differences in post-transcriptional modifications that may be specific to a given cell type, such as splicing, should not be limiting by the use of only a single type of mature mRNA (Table 1) [37–40].

TiPeR of selected transcription factor mRNAs has also been used to generate a pluripotent stem cell. The recent report from the Givol group using mRNA derived from four transcription factors used to create iPS cells was the first to demonstrate the successful RNA-mediated generation of iPS clones from fibroblasts [39]. This was followed by similar reports from Warren et al. and Plews et al. that further confirm the idea of RNA mediated cellular reprogramming to generate stem cells [38, 41]. The Warren et al. paper used chemically modified mRNA that presumptively increases the cellular half-life of the transfected RNAs but it should be noted that this modification is not necessary for the functioning of the mRNAs or to create phenotypic conversion or to maintain cell viability. Many studies have demonstrated that the transfection of unmodified mRNAs, and unmodified small interfering RNAs were functional without apparent drawbacks [15, 16, 39, 42, 43] [44]. Technically, it may be of note that adding a poly-A tail and capping the *in vitro* transcribed mRNA may improve the efficiency of translation and stability of mRNA but any modifications should be examined experimentally to verify the effects of modifications. As the RNA mediated induction of stem cell is distinct from the DNA-mediated method and involves two or more RNAs, it should be designated as transcriptome mediated pluripotent stem cell (tPS).

iPS cells have also been produced by performing TiPeR of fibroblasts using a specific miRNA [40]. This observation further suggests the importance of the dynamic expression state of a cell as a dominant determinant of cell phenotype in as much as miRNAs work to generally suppress expression of either gene transcription or protein-translation. Whereas mRNA TiPeR generally produces a gain of function (increased protein production) miRNA TiPeR would produce a loss of function (decrease in protein production). miRNAs act in a linear manner meaning that an individual miRNA will bind to one target site and exert its inhibitory function. As there are generally multiple binding sites for any particular miRNA in several mRNAs of the expressed transcriptome, the miRNA will act to inhibit the expression of several mRNAs (Figure 3).

The success of TiPeR of both mRNAs and miRNAs in altering the phenotype of cells can be viewed in context of the Waddington's original epigenetic landscape theory [45]. However, TiPeR shows that this epigenetic landscape can be considered more as a barrier to keep the cell within a phenotypic range of common expression, and can be modified through the addition of exogenous elements. Thus, a single cell's specific epigenetic landscape contains common factors shared across a given cell type (eg neuron, cardiomyocyte) as well as an individual transcriptome signature with a unique frequency and amplitude. The success of cell type conversion will be dependent on the strength of exogenous forces to modulate those variable elements to ease the TiPeR process. mRNA TiPeR would increase the amount of selected functional mRNAs and proteins altering their cellular ratios in an effort to go over the epigenetic barriers to transdifferentiation. In conjunction with this action, miRNA TiPeR can be thought of as through inhibition of mRNA and protein production as lowering of the epigenetic barrier to transdifferentiation. The result of either process would be the transdifferentiation of the host cell into the destination cell. It is likely that future TiPeR mediated transdifferentiation will exploit the use of both gain of function (mRNA) and loss of function (miRNA) constituents to exert finer control over the transdifferentiation process.

The TiPeR technology is in its infancy hence there are still many unanswered questions including - how do the mRNAs that produce a particular transdifferentiation event compare

with the mRNAs that are inhibited by miRNAs that produce the same transdifferentiation? Also does one need more molecules of a miRNA to elicit a phenotypic change as compared to mRNAs that would create the same destination phenotype? These are fundamental questions that bear on how cells develop and the pathways through which they can be manipulated to produce the desired phenotype. The importance of such questions and the ease in the use of RNAs for transdifferentiation highlights the advantages of direct RNA use, as opposed to DNA driven expression (Box 1).

Future directions and conclusions

To achieve high efficiency direct somatic cell phenotype remodeling, there needs to be extensive understanding of the quantitative aspects of the specified cells phenotypic status. In general the most important phenotypic marker to consider is the functionality of the cell. The phenotype of cells after differentiation is a dynamic state resulting in balancing of functional proteins and RNAs to maintain homeostasis of specific functional phenotype. The success of RNA and DNA mediated cellular phenotypic conversions demonstrates that breaking proteomic homeostasis in terminally differentiated cells through the expression of selected RNAs can produce cellular reprogramming regimens. The efficiency of this procedure not only depends on the efficiency or introduction of the mediators (e.g. transcription factors for iPS) but also likely relies on the cells phenotypic status. This status as dictated by the cellular expression profile can be thought of as the phenotypic or cellular memory. This cellular memory can be represented as a 3-dimensional space bounded by the expression profiles of all cells of a particular phenotypic status. For a cell near the shell edge it may be possible for this cell to be more easily modified by TIPeR into a distinct cell type than one on the opposite edge of the same phenotypic shell. In addition to the uncertainty of determining a precise site of insertion, the copy number, and the efficiency of DNA-mediated insertion of transcription factors, a cell's initial phenotypic status will play an important role in the reprogramming process. Transcriptome mediated remodeling utilizes a transcriptome generated from a donor cell culture or multiple RNAs that have been mixed in selected ratios. Pooling the transcriptomes from many cells of the same type will limit the effect of single cell variation, as transfection of a homogeneous transcriptome into acceptor cells reduces the variability originating from multiple transfections introducing heterogeneous single cell transcriptomes. The method of transcriptome mediated cell remodeling utilizes the innate proteomic and biochemical enzyme system of the host cell as the machinery for remodeling, in contrast to the stereotyped, iterative, reprogramming that is necessary in transcription factor stimulated iPS development. Although this may delay the immediate conversion of a cell to another type, the principles underlying the TIPeR process have a clear benefit in therapeutic applications as it opens up the possibility of on-site, direct remodeling of target cells in tissue without first isolating the cells. This possible tissue specific use of TIPeR has another potential benefit as cells undergoing changes *in vivo* will be subject to epigenetic and environmental influences from surrounding healthy tissue.

As TIPeR may refer to either whole transcriptome or partial transcriptome transfer into a host-cell it is important to note the advantages and disadvantages of each. The advantage of using the whole transcriptome to elicit phenotypic conversion is that the donor RNAs are present in the ratios and amounts required for the sought after phenotype to function. This is an unbiased approach to generation of cellular phenotype. Further there may be specific splice variants, RNA-edited forms of the endogenous RNA or endogenous poly-A tail lengths, that are required for proper expression and phenotypic conversion that would not be available from available transcribable cDNA clones. The primary limitation of this approach is that a source of the whole transcriptome will be necessary for transfer and such sources (especially for human phenotypic transfers) may be limiting. On the other hand, use of defined selected RNAs that will induce phenotypic remodeling have the advantage of being

in vitro transcribed from cDNA clones so there is no limit to the amount available. However, the limitation of knowing the exact mRNAs necessary to elicit phenotypic remodeling and the lack of knowledge of the endogenous mRNA modifications may make this approach problematic for some applications. However, with these limitations in mind, it is clear that with the ability to more rapidly and completely (e.g all splice forms of RNAs within a cell) characterize cellular transcriptomes by NextGen sequencing, it will be advantageous to develop approaches that better permit selection of defined RNAs (and their specific functional forms) not simply transcription factors but also other perhaps more subtle modulators of phenotype, for use in TIPeR-mediated phenotypic conversions.

Future development of the TIPeR process will focus on identifying the subset of mRNAs, which define functional phenotypic homeostasis as well as mRNAs and miRNAs which work to maintain an epigenetic barrier to phenotypic conversion. These sets of mRNA work both as a “cellular phenotypic memory” to maintain and sustain an acceptable range of phenotypes in differentiated cells and to direct a lineage pathway through the process of development. When this homeostasis is perturbed, the cell may enter a different phenotypic state, which if inappropriately controlled may lead to production of a dying cell or alternately permit a cell to enter an oncogenic phase. Instead of introducing an excess amount of RNA, one can put specified amounts of any single or multiple RNAs in order to modulate the ratio of mRNAs to generate/maintain a given phenotype. In this manner the expression profile of a desired cell type can be placed into a host cell with the goal of this specific expression profile eliciting that phenotype. It is also important to note that as RNA is the inducer in the TIPeR approach, that the RNA will degrade under normal cellular conditions thereby removing the inducer thereby overcoming any issues with aberrant constant overexpression caused by promoter driven DNA constructs. Finally, it is also possible to repeatedly TIPeR a cell so that the amount of RNA and their ratios can be altered in a defined manner to insure that high enough levels of RNA are present to induce phenotype remodeling but also varying the ratios of RNA to more closely resemble developmental changes that may facilitate chromatin remodeling and phenotype emergence. Using TIPeR, it is possible to take advantage of the ability to modify homeostasis and overcome cellular epigenetic barriers. With this approach, the addition of miRNA should lower the epigenetic barrier and allow the total amount of inductive mRNA to be reduced. Further, by more selectively adding mRNA specifically involved with the generation of a given phenotype, the ability to directly phenoconvert with a minimum number of RNA transfections will be increased.

Finally given the ease with which cell phenotype can be converted by alteration of a cells expression profile, it is possible and perhaps likely that endogenous cells undergo phenotypic conversion in response to particular stimuli. Such stimuli may include viral infection of cells where the introduced virus modulates host cell gene expression to produce a distinct cell type or perhaps introduction of RNAs into cells from released endosomes from other cells that then function in the host cell to modify phenotype. While there is scant but accumulating evidence that this may indeed be the case it is reasonable to investigate the underlying process of RNA memory stabilization of phenotype as it may prove to be an effective way of manipulating endogenous cells and therefore provide a novel but important therapeutic area for drug discovery.

Box 1. Considerations in RNA mediated methodology

RNA advantages

- No vector required –expression through *in vivo* transcription.

- Can add specific and known amounts of RNA –no need for promoter driven regulation.
- No need for the RNA to go to the nucleus (as for vector mediated transfer) can be directly used in the cell cytoplasm.
- Can be directed to specific subcellular sites where activity can be enhanced.
- RNA is transient so exogenous influence is removed –no long term deleterious effect of added reagent.
- Can add multiple RNAs in specified ratios –can position anywhere in phenotype shell.
- Allows the use of RNA populations with edited and spliced forms of RNAs that may be necessary for conversion.
- Process can be repeated until desired phenotype is reached.
- No integration to the genome, no DNA break or damage, no danger of malignancy as is the case with factors of DNA.

Potential RNA disadvantages

- RNA may need to be enzymatically capped and poly-adenylated to increase intracellular translational efficiency.
- RNA addition may need to be repeated multiple times.
- May need to inhibit host transcription transiently to facilitate TIPeR.

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Glossary Box

Totipotency	The ability of cell to produce all types of differentiated cells.
Pluripotency	The ability of a stem cell to generate three germ layers, endoderm, mesoderm and ectoderm.
SNT or SCNT (Somatic Cell Nuclear Transfer)	A technique to create a cloned embryo by transferring donor nucleus into enucleated egg cell.
Transdifferentiation/ phenotype conversion	A biological process that transforms already differentiated cell into another differentiated cell type.
iPS cell (Induced Pluripotent Stem cell)	iPS cells are artificially-induced pluripotent cells derived from non-pluripotent cells by introduction of high levels of exogeneous transcription factors.. The most commonly used transcription factors are Oct4, Sox2, Klf4, c-Myc and Nanog.
ES cell (Embryonic Stem cell)	ES cells are pluripotent cells derived from the inner mass of a blastocyst in an early embryo.

TiPeR (Transcriptome Induced Phenotype Remodeling)	A methodology that converts one cellular phenotype to another cellular phenotype by introducing multiple RNAs into host cell.
tAstrocyte (transcriptome-effected Astrocyte)	tAstrocytes are astrocyte-like cells derived nonastrocyte cells by TiPeR methodology.
tCardiomyocyte (transcriptome-effected Cardiomyocyte)	tCardiomyocytes are cardiomyocyte-like cells derived from noncardiomyocyte cells by TiPeR methodology.
tPS cell (TiPeR Pluripotent Stem cell)	tPS cells are induced pluripotent stem cells generated from RNAs encoding or containing pluripotency inducing factors.

References

1. Gurdon JB, et al. Sexually mature individuals of *Xenopus laevis* from the transplantation of single somatic nuclei. *Nature*. 1958; 182:64–65. [PubMed: 13566187]
2. Wilmut I, et al. Viable offspring derived from fetal and adult mammalian cells. *Nature*. 1997; 385:810–813. [PubMed: 9039911]
3. Campbell KH, et al. Sheep cloned by nuclear transfer from a cultured cell line. *Nature*. 1996; 380:64–66. [PubMed: 8598906]
4. Campbell KH. A background to nuclear transfer and its applications in agriculture and human therapeutic medicine. *J Anat*. 2002; 200:267–275. [PubMed: 12033731]
5. McCulloch EA, Till JE. The radiation sensitivity of normal mouse bone marrow cells, determined by quantitative marrow transplantation into irradiated mice. *Radiat Res*. 1960; 13:115–125. [PubMed: 13858509]
6. Jiang Y, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature*. 2002; 418:41–49. [PubMed: 12077603]
7. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006; 126:663–676. [PubMed: 16904174]
8. Chang CW, et al. Polycistronic lentiviral vector for "hit and run" reprogramming of adult skin fibroblasts to induced pluripotent stem cells. *Stem Cells*. 2009; 27:1042–1049. [PubMed: 19415770]
9. Heng JC. The nuclear receptor Nr5a2 can replace Oct4 in the reprogramming of murine somatic cells to pluripotent cells. *Cell Stem Cell*. 6:167–174. [PubMed: 20096661]
10. Huangfu D, et al. Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. *Nat Biotechnol*. 2008; 26:1269–1275. [PubMed: 18849973]
11. Ichida JK, et al. A small-molecule inhibitor of TGF- β signaling replaces Sox2 in reprogramming by inducing Nanog. *Cell Stem Cell*. 2009; 5:491–503. [PubMed: 19818703]
12. Yu J, et al. Human induced pluripotent stem cells free of vector and transgene sequences. *Science*. 2009; 324:797–801. [PubMed: 19325077]
13. Ieda M, et al. Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. *Cell*. 2010; 142:375–386. [PubMed: 20691899]
14. Zhou Q, et al. In vivo reprogramming of adult pancreatic exocrine cells to β -cells. *Nature*. 2008; 455:627–632. [PubMed: 18754011]
15. Sul JY, et al. Transcriptome transfer produces a predictable cellular phenotype. *Proc Natl Acad Sci U S A*. 2009; 106:7624–7629. [PubMed: 19380745]
16. Kim TK, et al. Transcriptome transfer provides a model for understanding the phenotype of cardiomyocytes. *Proc Natl Acad Sci U S A*. 2011; 108:11918–11923. [PubMed: 21730152]
17. Stadtfeld M, Hochedlinger K. Induced pluripotency: history, mechanisms, and applications. *Genes Dev*. 2010; 24:2239–2263. [PubMed: 20952534]

18. Beyhan Z, et al. Transcriptional reprogramming of somatic cell nuclei during preimplantation development of cloned bovine embryos. *Dev Biol.* 2007; 305:637–649. [PubMed: 17359962]
19. Munsie MJ, et al. Isolation of pluripotent embryonic stem cells from reprogrammed adult mouse somatic cell nuclei. *Curr Biol.* 2000; 10:989–992. [PubMed: 10985386]
20. Mertes H, Pennings G. Cross-border research on human embryonic stem cells: legal and ethical considerations. *Stem Cell Rev.* 2009; 5:10–17. [PubMed: 19052926]
21. Wernig M, et al. Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. *Proc Natl Acad Sci U S A.* 2008; 105:5856–5861. [PubMed: 18391196]
22. Zhang J, et al. Functional cardiomyocytes derived from human induced pluripotent stem cells. *Circ Res.* 2009; 104:e30–e41. [PubMed: 19213953]
23. Gore A, et al. Somatic coding mutations in human induced pluripotent stem cells. *Nature.* 2011; 471:63–67. [PubMed: 21368825]
24. Stadtfeld M, et al. Induced pluripotent stem cells generated without viral integration. *Science.* 2008; 322:945–949. [PubMed: 18818365]
25. Okita K, et al. Generation of mouse-induced pluripotent stem cells with plasmid vectors. *Nat Protoc.* 2010; 5:418–428. [PubMed: 20203661]
26. Okita K, et al. Generation of mouse induced pluripotent stem cells without viral vectors. *Science.* 2008; 322:949–953. [PubMed: 18845712]
27. Kane NM, et al. Lentivirus-mediated reprogramming of somatic cells in the absence of transgenic transcription factors. *Mol Ther.* 2010; 18:2139–2145. [PubMed: 20978477]
28. Okita K, et al. Generation of germline-competent induced pluripotent stem cells. *Nature.* 2007; 448:313–317. [PubMed: 17554338]
29. Elkabetz Y, et al. Human ES cell-derived neural rosettes reveal a functionally distinct early neural stem cell stage. *Genes Dev.* 2008; 22:152–165. [PubMed: 18198334]
30. Ben-Porath I, et al. An embryonic stem cell-like gene expression signature in poorly differentiated aggressive human tumors. *Nat Genet.* 2008; 40:499–507. [PubMed: 18443585]
31. Chiou SH, et al. Positive correlations of Oct-4 and Nanog in oral cancer stem-like cells and high-grade oral squamous cell carcinoma. *Clin Cancer Res.* 2008; 14:4085–4095. [PubMed: 18593985]
32. Han DW, et al. Epiblast stem cell subpopulations represent mouse embryos of distinct pregastrulation stages. *Cell.* 2010; 143:617–627. [PubMed: 21056461]
33. Han DW, et al. Direct reprogramming of fibroblasts into epiblast stem cells. *Nat Cell Biol.* 2011; 13:66–71. [PubMed: 21131959]
34. Hanna J, et al. Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. *Science.* 2007; 318:1920–1923. [PubMed: 18063756]
35. Zhao T, et al. Immunogenicity of induced pluripotent stem cells. *Nature.* 2011; 474:212–215. [PubMed: 21572395]
36. Kim J, Eberwine J. RNA: state memory and mediator of cellular phenotype. *Trends Cell Biol.* 2010; 20:311–318. [PubMed: 20382532]
37. Kim D, et al. Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. *Cell Stem Cell.* 2009; 4:472–476. [PubMed: 19481515]
38. Warren L, et al. Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. *Cell Stem Cell.* 2010; 7:618–630. [PubMed: 20888316]
39. Yakubov E, et al. Reprogramming of human fibroblasts to pluripotent stem cells using mRNA of four transcription factors. *Biochem Biophys Res Commun.* 2010; 394:189–193. [PubMed: 20188704]
40. Anokye-Danso F, et al. Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency. *Cell Stem Cell.* 2011; 8:376–388. [PubMed: 21474102]
41. Plews JR, et al. Activation of pluripotency genes in human fibroblast cells by a novel mRNA based approach. *PLoS One.* 2010; 5:e14397. [PubMed: 21209933]
42. Job C, Eberwine J. Identification of sites for exponential translation in living dendrites. *Proc Natl Acad Sci U S A.* 2001; 98:13037–13042. [PubMed: 11606784]

43. Aakalu G, et al. Dynamic visualization of local protein synthesis in hippocampal neurons. *Neuron*. 2001; 30:489–502. [PubMed: 11395009]
44. Malone RW, et al. Cationic liposome-mediated RNA transfection. *Proc Natl Acad Sci U S A*. 1989; 86:6077–6081. [PubMed: 2762315]
45. Waddington, CH. *The evolution of an evolutionist*. Cornell University Press; 1975.

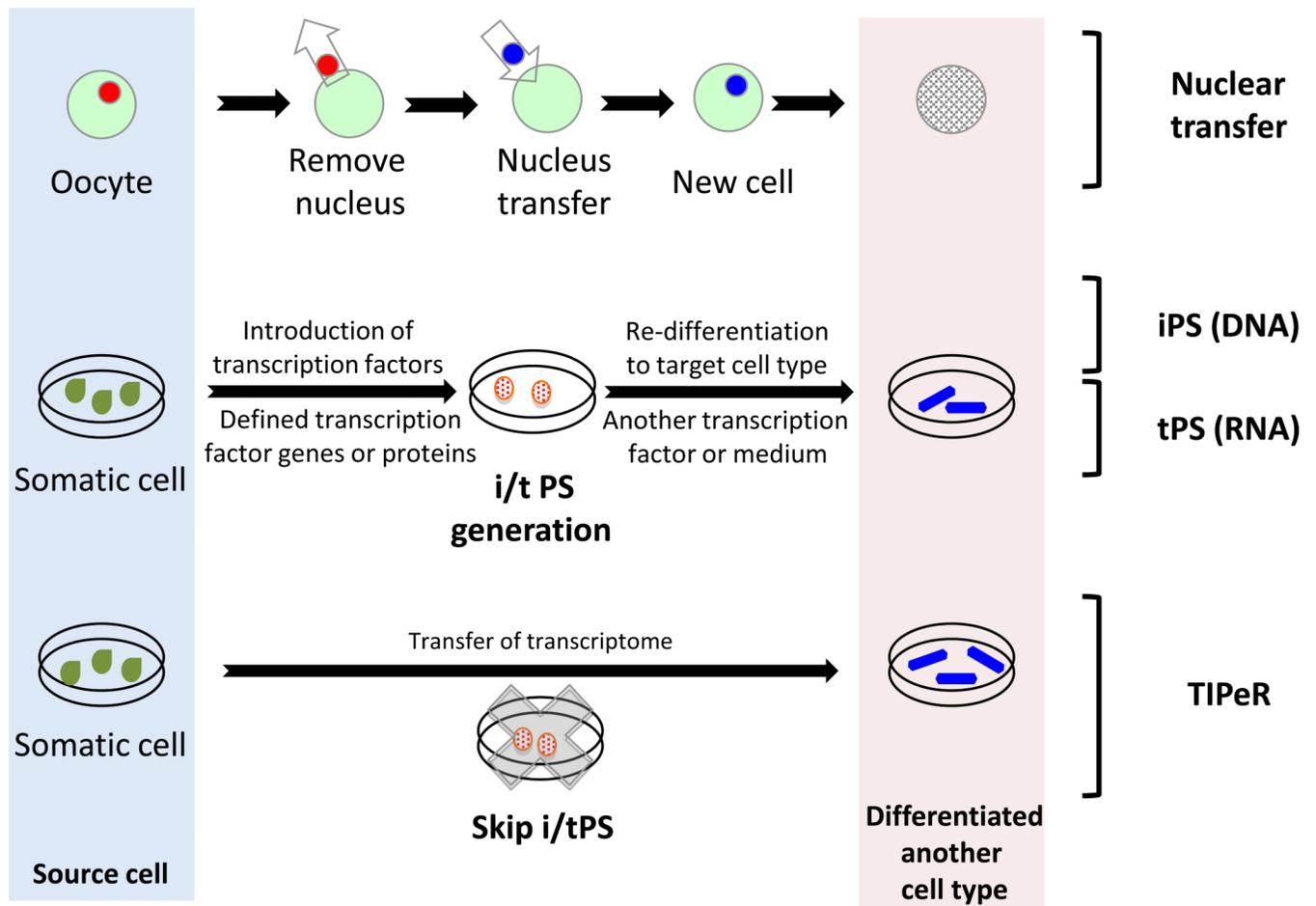


Figure 1.

A schematic of three different cellular reprogramming methods. Somatic nuclear transplant transfers a whole nucleus from a cell into an enucleated fertilized oocyte (Top). The introduction of defined transcription factors dedifferentiates a somatic cell to a pluripotent stem cell (iPSC) which redifferentiates into a new cell type of interest using specific differentiation media or other transcription factors (Middle). TIPeR methodology skips intermediate iPSC generation and directly transdifferentiates a source cell into a target cell via transfer of the transcriptome (mRNA) (Bottom). For TIPeR, the whole transcriptome is extracted from source cells and poly-A⁺ RNAs are isolated. The isolated poly-A⁺ RNAs are transfected into recipient cell to induce transdifferentiation.

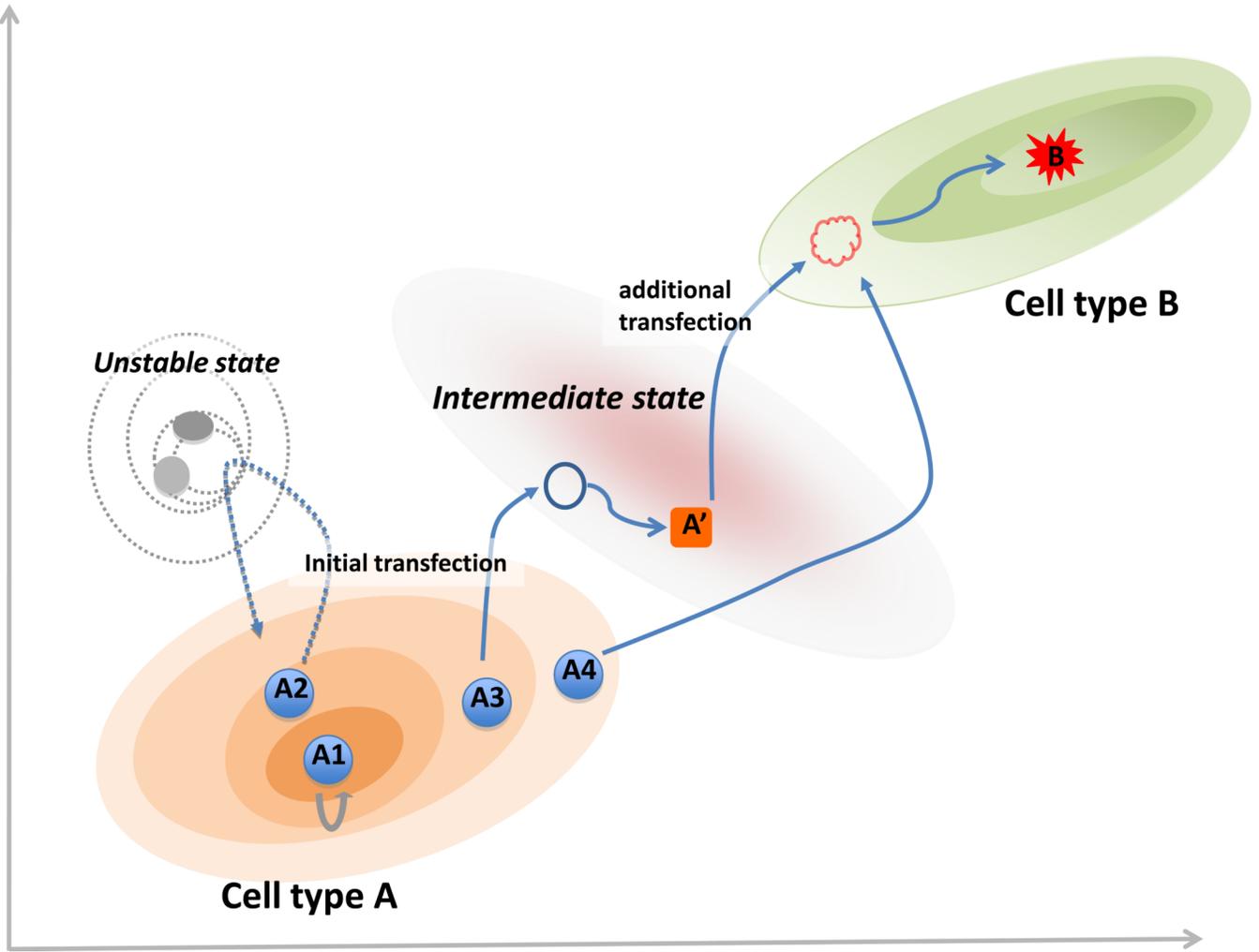


Figure 2.

A schematic illustration of TIPeR conversion. The effect of transcriptome transfer is modulated by the initial cell state. The transcriptomes of individual cells of type A (A1–A4) are modified through an initial transfection. The transfected cells will either resist phenotype change (A1), shift status to a transient unstable state and return to the original state (A2) or escape the stable state (A3, A4). The escaped cells may enter an intermediate state (open blue circle or A' in the pink cloud) or may shift phenotype to the edge of cell type B and then spontaneously converted into phenotype (B). A second introduction of transcriptome to A' facilitates the transdifferentiation of A' located in intermediate state to cell type B.

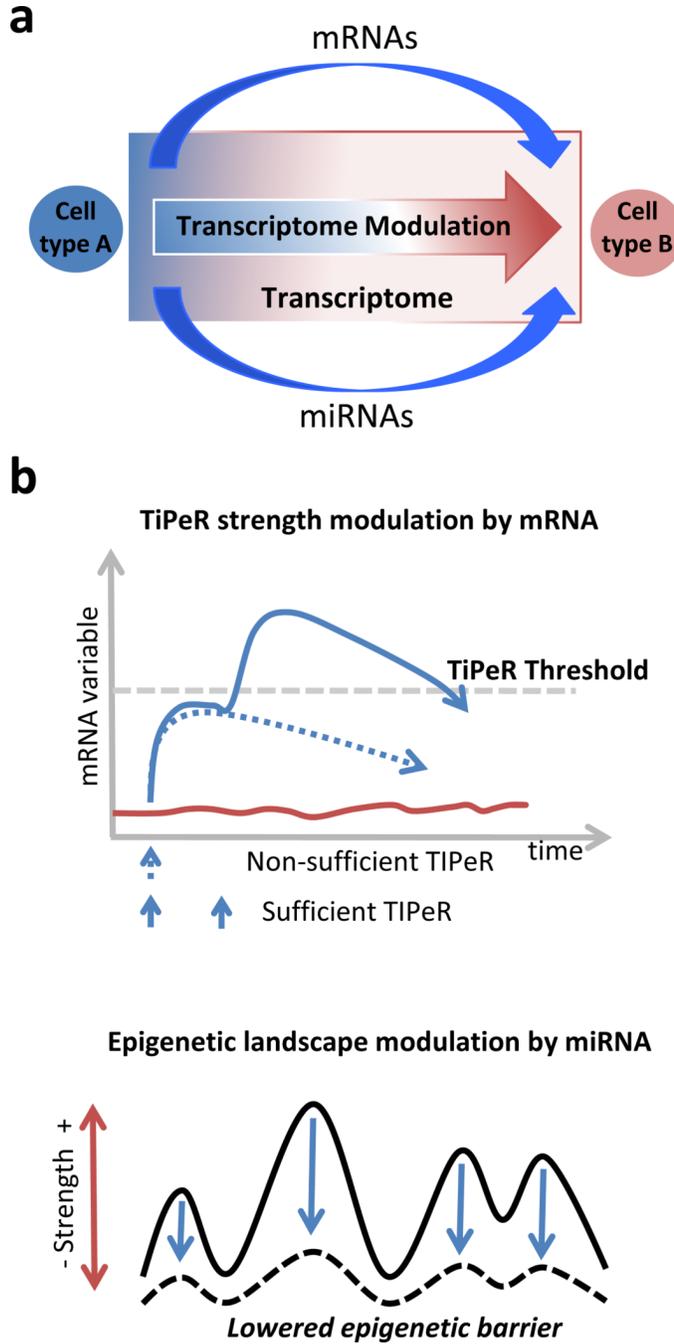


Figure 3. Transcriptome modulation is regulated by both mRNA and miRNA. (a). The addition of mRNAs drives transdifferentiation towards a target transcriptome while the addition of miRNA suppresses the source 480 transcriptome. (b) A single introduction of mRNA may not be sufficient to reach the required threshold for phenotype transformation (TiPeR Threshold). A repeated cycle of TiPeR (Upper) would allow the cell to pass the threshold. Alternately, the introduction of miRNA could lower the epigenetic landscape barrier (Lower panel), consequently enhancing TiPeR efficiency. The strength of cellular phenotypic memory can be modulated by the amount of mRNA or miRNA applied as well as the length of time the mRNA or miRNA is available for the phenotype conversion process.

Table 1Phenotype transfer and cellular reprogramming summary^a

Year	Factors	Delivery	Source Cell Type	Reprogrammed to	Species	Ref
2009	miRNA	Phototransfection	Neuron	tAstrocyte	Rat	[15]
2009	Protein(OKSM)	CPP	Fibroblast	iPS	Human	[37]
2010	mRNA(OKSM)	Lipid-mediated	Fibroblast	iPS	Human	[39]
2010	mRNA(OSLN)	Lipid-mediated	Fibroblast	iPS	Human	[38]
2011	miRNA	Virus	Fibroblast	iPS	Mouse	[40]
2011	mRNA	Lipid-mediated	Fibroblast Astrocyte	tCardiomyocyte tCardiomyocyte	Mouse	[16]

^a Abbreviations: O:Oct3/4, K:Klf4, S:Sox2, M:c-Myc, L:Lin28 and N:Nanog