Published in final edited form as:

Mol Biosyst. 2013 January 27; 9(1): 10–17. doi:10.1039/c2mb25088h.

Learning the molecular mechanisms of the reprogramming factors: let's start from microRNAs

Chao-Shun Yang and Tariq M Rana*

Program for RNA Biology, Sanford-Burnham Medical Research Institute, La Jolla, California 92037, USA

Abstract

Induced reprogramming of somatic cells has had a great impact on stem cell research, and the reprogramming technologies have evolved from four transgenic factors (Oct4, Sox2, Klf4, and c-Myc; OSKM) to just a few microRNAs (mainly miR-290/302 seed family). Despite these advances, the molecular events occurring during various stages of reprogramming remain largely unknown. Here, we concisely review current knowledge of miRNA regulation from the initiation phase of OSKM-induced reprogramming, through the transitional stage, to final maturation. At the start of reprogramming, the microRNAs miR-21, miR-29a, let-7a, and miR-34 act as guards to secure the somatic identity and genomic integrity of the cell of origin. As reprogramming proceeds, miR-155, miR-10b, miR-205, and miR-429 modulate the epithelial-mesenchymal/mesenchymal-epithelial transition (EMT/MET), which is a critical step towards transformed pluripotent status. Finally, the pluripotency regulatory network is secured in the iPSCs and fine-tuned by a group of miRNAs belonging to the miR-290/302 seed family. Among the four reprogramming factors, c-Myc plays the dominant role in regulating the miRNAs under reprogramming-specific conditions. Accumulating evidence suggests that the reprogramming efficiency can be improved by either blocking barrier miRNAs or introducing helper miRNAs. Intriguingly, induced pluripotency can be obtained by introducing a single miR-302 cluster, although the supportive molecular mechanism is still lacking. In the near future, we may be able to realize the broad potential of miRNAs in the stem cell field, such as altering cell identities with high efficiency through the transient introduction of tissue-specific miRNAs.

Induced reprogramming overview

The new era of reprogramming was initiated by the ectopic expression of four transcription factors in somatic cells, first demonstrated in mouse cells¹ and later in human cells^{2–6}, which have the capacity to differentiate into different cell lineages. Using retroviral or lentiviral systems, these four factors, Oct4, Sox2, Klf4/Lin28, and c-Myc/Nanog (also referred to as OSKM or OSLN) can be easily introduced into somatic cells to induce reprogramming to an embryonic stem (ES) cell-like pluripotent state. The induced pluripotent stem cells (iPSCs) generated by this breakthrough technology have provided a valuable alternative resource to human embryonic stem cells⁷. However, the low efficiency of reprogramming and concerns of genetic modification by the transgenes remain major hurdles in the therapeutic application of iPSCs^{2, 4, 7, 8}. In recent years, substantial progress has been made in improving reprogramming efficiency and in substituting select transcription factors^{8–11}. A few reports have also revealed the great promise of inducing reprogramming with only mRNAs or microRNAs (miRNAs)^{12–16}. Although many windows have been opened to improve the efficiency of reprogramming and to minimize transgenic integrations into the

*Corresponding author Program for RNA Biology, Sanford-Burnham Medical Research Institute, 10901 North Torrey Pines Road, La Jolla, CA 92037, USA. Fax: + 1 858 795 5328; Tel: + 1 858 795 5325; trana@sanfordburnham.org.

genome, we have only just begun to understand the molecular mechanisms that control reprogramming beyond the four factors. Many studies have shown that reprogramming can be defined and achieved as a step-wise process¹⁷⁻¹⁹. Furthermore, several genes and proteins have been identified that have greatly impacted reprogramming efficiency, such as PTGS2²⁰, Ink4a/ARF, p53/p21²¹⁻²⁶, TGF- β ^{27, 28}, and miRNAs²⁹⁻³⁷.

MicroRNAs are ~22 nucleotide small non-coding RNAs that are highly conserved among species^{38, 39}. They contain short sequences in the 5' end ("seed" regions) that direct target gene recognition of miRNA-loaded processing complexes, RISCs (RNA-induced Silencing Complexes)⁴⁰. In mammals, miRNAs act as post-transcriptional regulators to reduce translation of target genes by either destabilizing mRNAs or blocking their translation. miRNAs have been shown to play critical roles in various physiological processes, including embryogenesis⁴¹⁻⁴³ and tumorigenesis⁴⁴⁻⁴⁸. In addition, numerous reports have shown that miRNAs play significant roles in somatic cell reprogramming to iPSC^{29-35, 49}. The progress and expectations of induced reprogramming technology have been recently described in numerous review articles^{8-10, 50-57}. Several reviews^{42, 43, 58-64} also address the improvement of reprogramming methods by introducing miRNAs upon induced reprogramming. However, intrinsic roles of miRNAs, which are regulated by OSKM at each stage of reprogramming process, have not been addressed. In this review, we discuss the molecular mechanisms of reprogramming from this unique viewpoint, focusing on the effects of the reprogramming factors on endogenous miRNA regulation and the regulatory networks of these miRNAs during iPSC induction.

Reprogramming is a stochastic but step-wise event

Reprogramming is induced by ectopic expression in somatic cells of the four reprogramming factors that drive the cells to de-differentiate and achieve a state of pluripotency. An increasing body of evidence shows that it is a generally stochastic manner⁶⁵⁻⁶⁷ but is able to achieve step-wise transition during reprogramming^{17-19, 65}. The OSKM reprogramming factors bind their targets in a coordinated fashion¹⁹ to initiate the first step of reprogramming, the transcriptional and epigenetic changes^{19, 68}. Furthermore, it has been suggested that OSKM may assemble an inhibitory circuit against somatic identity prior to building up the transcriptional network of pluripotency in the later stages of the transition^{19, 68}. This observation is supported by other reports showing that a number of barriers need to be overcome to reach the next steps in the transition^{9, 10}.

c-Myc plays a key role in establishing the early transition stage

The cellular phenotypes associated with the reprogramming transitions have been reported in recent studies^{69, 70}, but a clear picture of the detailed molecular events driving the transitions is still lacking. Among the four reprogramming factors, c-Myc has been shown to play the dominant role in initiating the early transitional stage^{19, 68}. Expression of c-Myc alone can downregulate the expression of fibroblast-specific genes and induce the molecular context of the embryonic status within 3 days of transduction¹⁹. In addition to regulating the expression of hundreds of genes, as shown in previous reports^{19, 71}, c-Myc regulates numerous miRNAs to promote tumorigenesis⁷²⁻⁷⁵ and to maintain pluripotency in ES cells⁷⁶⁻⁷⁹. Recently, we demonstrated that c-Myc disrupts the fibroblastic network by inhibiting the mouse embryonic fibroblast (MEF)-enriched miRNAs, miR-21 and miR-29a (Figure 1), to lower the threshold for reprogramming²⁹. Thus, c-Myc establishes the early molecular context of reprogramming, not only by directly interacting with promoter regions of target genes, but also by exerting inhibitory effects on somatic networks by regulating miRNAs.

The MEF-enriched miRNAs, miR-21, miR-29a, and let-7, act as barriers to the initial stage of reprogramming

We previously demonstrated that the miRNA expression profile changes dramatically upon OSKM introduction into MEFs, with c-Myc playing the dominant regulatory role in this process²⁹. Furthermore, we have shown that c-Myc decreases the expression of MEF-enriched miRNAs, such as miR-21 and miR-29a (Figure 1). c-Myc transcriptionally suppresses miR-29 expression by binding to its promoter⁸⁰, while the molecular mechanism by which c-Myc regulates miR-21 expression is still unclear. miR-21 positively regulates the TGF β ¹⁸¹ and MAP kinase⁸² pathways, which have been shown to act as roadblocks to reprogramming^{27, 28, 31, 34, 69, 83-86}. miR-29a has been shown to indirectly induce p53 protein levels by post-transcriptionally inhibiting CDC42 and p85 α ⁸⁷. Consistent with these observations, depleting miR-21 or miR-29a dramatically (2 to 3 fold) increases reprogramming efficiency, suggesting that MEF-enriched miRNAs act as barriers to reprogramming²⁹. We also showed that miR-21 and miR-29a modulate reprogramming by regulating phosphorylation of ERK1/2 by 45~60% through Spry1 protein expression. In addition, depletion of miR-21 and miR-29a downregulates p53 protein levels by 25~40% through elevation of CDC42/p85 α expression, which consequently enhances reprogramming efficiency. These data provide evidence for new regulatory networks during reprogramming involving c-Myc, miR-21, and miR-29a.

Another abundant miRNA in MEFs, let-7 (Figure 1), has been shown to act as a barrier to reprogramming, since depleting let-7 enhanced the reprogramming efficiency by 4.3 fold with only the OSK reprogramming factors³². Ectopic expression of c-Myc reduces let-7a expression in MEFs during reprogramming, although to a relatively modest degree²⁹. It has been reported that c-Myc represses let-7 through Lin-28b transactivation⁷⁵; however, Lin-28b mRNA is undetectable during the early stage of reprogramming²⁹, suggesting other indirect mechanisms may be involved. The let-7 family may exert negative effects on reprogramming, because they are known to repress numerous pluripotent regulators, including Myc, Hmga2, Lin-28, and Sall4^{32, 88-91}. To summarize, the MEF-enriched miRNAs, miR-21, miR-29a, and let-7a modulate various pathways to antagonize the reprogramming process. Furthermore, c-Myc has an intrinsic ability to initiate the reprogramming transition, not only by targeting the promoter regions of numerous genes, but also by inhibiting MEF-enriched miRNAs in the initial stage of reprogramming.

p53-regulated miRNA miR-34 and miR145 play important roles in reprogramming

The introduction of reprogramming factors into somatic cells initiates the cellular stress response to viral infection and oncogenes. Among the stress response molecules, transformation-related protein 53 (Trp53 or p53) plays a critical role as a gate-keeper to ensure that only cells with genomic integrity will survive to reach the pluripotent status, while stochastic nuclear reprogramming is induced²¹⁻²⁶. miR-34 (Figure 1) has been identified as a downstream target of p53 and contributes significantly to p53-mediated cell cycle arrest and apoptosis⁹²⁻⁹⁴. miR-34a deficiency in murine somatic cells improves the efficiency (> 4 fold) and kinetics (by two days) of reprogramming⁹⁵. Consistent with this observation, p53-induced miR-34a/b/c act as negative regulators of reprogramming, in part through the repression of pluripotency genes such as Lin28a⁹⁶, Nanog, Sox2, N-Myc⁹⁵, and c-Myc⁹⁷.

After initiation of reprogramming, mesenchymal-to-epithelial transition (MET) is the next step towards pluripotency^{69, 70}. Approximately 5 days after OSKM induction, transformed

cells undergo dramatic morphological changes from mesenchymal-like (polarized and mono-adherent) to epithelial-like (densely packed) cells. MET is critical for somatic cells to complete the first step of de-differentiation. Interestingly, miR-34a/b/c have been shown to compromise Snail1-dependent EMT (the reverse transition to MET) in cancer cells by targeting the 3' untranslated region (UTR) of Snail1⁹⁸, while Snail1 and ZEB1 impose a negative feedback loop on miR-34a/b/c by binding the E-boxes of the miR-34a/b/c promoters⁹⁷. miR-34a also decreases other EMT factors, such as β -catenin, LEF1, Axin2⁹⁸, Slug, and ZEB1⁹⁷, and ectopic expression of miR-34a also prevents TGF- β -induced EMT⁹⁷. Therefore, short-term introduction of miR-34a/b/c may suppress the EMT in the early reprogramming process, while reprogramming factors coordinately affect MET to de-differentiate somatic cells. Since miR-34 modulate various functional pathways, miR-34 may play dual roles to secure cell integrity and promote MET in the cell-context-dependent manner.

miR-145, suggested to be the direct target of p53⁹⁶, plays critical roles to direct differentiation of ES cells⁹⁹. miR-145 has been reported to downregulate Sox2¹⁰⁰, Klf4⁹⁶, and Oct4 to promote mesoderm and ectoderm differentiation in ES cells⁹⁹. Furthermore, miR-145 promoter region is bound and repressed by Oct4 in ES cells⁹⁹, suggesting Oct4 may lift the suppression of endogenous OSK by miR-145 during reprogramming. However, regulatory networks of miR-145 during reprogramming need to be vigorously interrogated, since this hasn't been tested during the process of reprogramming.

EMT/MET-associated miRNAs, miR-155, miR-10b, miR-205, and miR-429 play important roles in modulating the transitional stage of reprogramming

During the MET stage of reprogramming (Figure 1), pro-mesenchymal miRNAs miR-10b¹⁰¹ and miR-155¹⁰² decrease⁶⁹, and pro-epithelial miRNAs miR-205^{103, 104} and miR-429 (miR-200 family)^{49, 105-107} increase^{69, 70}. miR-10b promotes EMT in cancer metastases by targeting homeobox D10¹⁰¹, and miR-10b antagomirs suppress metastases in vivo¹⁰⁸. miR-155 plays an important role in TGF- β -induced EMT by targeting RhoA¹⁰², one of the key factors maintaining junction formation and stabilization¹⁰⁹. However, a recent report showed that miR-155 may have dual functions in modulating EMT depending on the microenvironment of the tumor¹¹⁰, suggesting that the functions of miRNAs are cell and/or tissue context-dependent.

Conversely, the miR-200 family and miR-205 have been shown to positively regulate MET by targeting ZEB1 and ZEB2^{107, 111-113}, while ZEB1 reciprocally represses the miR-200 family^{105, 106}. In addition, bone morphogenetic protein (BMP), which is required for efficient reprogramming with OSKM, promotes MET and also induces expression of miR-205 and the miR-200 family during OSKM-induced reprogramming⁷⁰. Introduction of miR-200b/c mimics synergize with OSKM to promote more efficient reprogramming⁷⁰. Notably, c-Myc (Figure 1) may boost reprogramming efficiency by directly inducing expression of the miR-200 family (miR-200, miR-141, and miR-429), and possibly coordinating with Klf4 to initiate MET^{69, 114}.

The miR-290/302 seed family plays significant roles during the programming progress

It has been shown that microRNAs maintain the murine ES property by promoting the G1-S transition of the cell cycle and that aberrant miRNA biogenesis impairs proliferation of ES cells, which accumulate in the G1 phase¹¹⁵⁻¹¹⁷. The key miRNAs for these functions are the miR-290 and miR-302 clusters, which are the most abundant miRNAs (Table 1) in mouse and human ES cells respectively¹¹⁸⁻¹²⁰. These two clusters have almost identical seed

regions (miR-290/302 seed family; also see Table 2), suggesting they have highly similar target and/or regulatory networks. In human ES cells, the miR-302 cluster is regulated by Oct4/Sox2 to post-transcriptionally modulate cyclin D1, a key regulator of cell cycle progression^{121, 122}. The miR-290/302 seed family modulates cell cycle progression by targeting diverse regulators of the cell cycle, including p21 and other inhibitors of the cyclin E/Cdk2 pathway¹¹⁵. Numerous targets of the miR-302 cluster were uncovered using the photoactivatable ribonucleoside-enhanced cross-linking and immunoprecipitation method (PAR-CLIP); these included modulation of BMP signaling through suppression of Tob2, Dazap2, and Slain1³⁰. miR-302a is known to target Lefty1/2, which is an agonist of the TGF- β /Nodal signaling pathway in embryogenesis¹²³. In mouse ES cells, the miR-290 family controls de novo methylation through Rbl2-dependent regulation of DNA methyltransferase (Dnmts)^{124, 125}. Based on these findings, it appears that the main function of the miR-290/302 seed family is to shorten the G1 phase of the cell cycle to support self-renewal, and to secure the epigenetic status that maintains the pluripotency of ES cells.

During MET stage of OSKM-induced reprogramming, aggregates and colonies of reprogrammed cells become visible under low magnification microscopy as reprogrammed cells acquire epithelial cell features. These cells begin to express pluripotency markers, of which SSEA-1 is the earliest surface marker to indicate the potential iPS cells^{17, 18}. As the reprogrammed cells move towards a state of pluripotency, Nanog, Esrrb, Lin28, Dppa4, Tert, Sox2, and Oct-4 are endogenously expressed, demonstrating that the core circuit of pluripotency has been established^{17-19, 70}. The embryonic stem cell cell-cycle-regulating (ESCC) miRNAs, the miR-302 clusters (Figure 1), are also expressed during the transition stage day 4 to day 7 post induced reprogramming^{34, 126}, mainly induced by the reprogramming factor c-Myc^{31, 37}. Recent study has shown that the promoter of miR-302 cluster can be bound and that the expression of miR-302 can be induced by vitamin C-dependent Oct4/Jhdm1b cooperation during reprogramming¹²⁷. The biogenesis of miRNAs has been shown to be critical to efficient reprogramming, because Ago2 downregulation reduces the number of iPSC colonies³⁴. Depletion of the miR-302 family reduces reprogramming efficiency in response to OSK or OSKM^{31, 127}, suggesting that the miR-302 family plays essential roles in the reprogramming process. Ectopic expression of the miR-290 or miR-302 clusters has been shown to improve OSKM- or OSK-reprogramming^{36, 37} by promoting MET through inhibition of the TGF- β receptor^{31, 34, 36}. Recent finding¹²⁸ also demonstrated that miR-290 cluster maintain pluripotency by repressing nuclear factor kappa B (NF- κ B) signaling pathway, which in turn restricts epithelial to mesenchymal transition in ES cells. Furthermore, the miR-290/302 seed family targets diverse functional groups to positively enhance induced reprogramming, including cell cycle regulation, (Cdkn1a, Rbl2, and Cdc216) and epigenetic regulation (Aof1, Aof2, Mecp1-p66, MECP2, Mbd2, and Smarcc2)^{13, 36}.

Other miRNAs have been identified to enhance reprogramming. For example, miR-17/92, miR-106b/25, and miR-106a/363 clusters boost reprogramming by targeting Tgfbr2 and p21³⁴. Notably, miR-17, miR-93, and miR-106a have also been induced during the MET stage (day 4 post reprogramming)^{34, 49}. Moreover, introduction of miR-106b and miR-93 miRNA mimics can promote MET (Figure 1) to boost reprogramming efficiency³⁴. The miR-130/301/721 family (Figure 1), identified by miRNA screening, target the homeobox transcription factor Meox2 (also known as Gax) to achieve ~2-fold increase in reprogramming³⁵. All those miRNAs (miR-17, 106a, 106b clusters, and miR-130/301/721 family) share a similar seed region with the miR-290/302 family (Table 2), suggesting that an abundance of miRNAs containing the miR-290/302 seed region play significant roles in various biological functions and intrinsically act as positive regulator to reprogramming.

The miR-290/302 seed family plays multiple roles at the ES stage¹²¹ and during the reprogramming process, and the miR-290 and miR-302 clusters are the predominant miRNA population expressed in mammalian ES cells. Therefore, the miR-290/302 seed family may have the potential to induce somatic cell reprogramming in the absence of transgenes OSKM. The reprogramming potential of the miR-302 cluster was first tested in human cancer cells, which showed that the signature properties of pluripotent stem cells are acquired in miR-iPSCs¹⁴. Following this finding, several reports demonstrated that the miR-302 cluster could reprogram various primary cell types into pluripotent stem cells^{13, 15, 16}. Among these, Anokye-Danso et al. demonstrated that miRNA-mediated reprogramming is more efficient than transcription factor-mediated method in both mouse (81.5% versus 17.9%) and human somatic cells (10% versus 0.004%)¹⁵. But to achieve this striking efficiency, suppression or low level of Hdac2 seems to be required in both mouse and human cells^{15, 16}. Miyoshi et al. further demonstrated that clinically-applicable iPS cells can be generated by introducing only a group of mature miRNAs (miR-200c, miR-302 family, and miR-369s), without retro-viral integration in genome¹⁶. However, this transfection-base reprogramming can only reach 0.01% reprogramming efficiency in mouse cells, and even lower (0.001%) in human cells¹⁶. The methodology of administrating miRNAs may be one of the main reasons to cause the difference in miR-induced reprogramming efficiency between these two reports, Anokye-Danso et al. (virus transduction)¹⁵ and Miyoshi et al. (small RNA transfection)¹⁶. In addition, distinct combinations of miRNAs were employed for reprogramming process: miR-302 cluster combined with valproic acid (VPA) treatment vs miR-200c, miR-302abcd, and miR-369s. Interestingly, VPA for Hdac2 suppression and miR-367 are required in viral transduction method, but both are dispensable in miR transfection method. These protocol variations may need to be vigorously tested to further improve the efficiency of reprogramming and clinical applicability of iPS cells. Despite the phenomenon of miR-induced somatic reprogramming, how microRNAs can perturb somatic molecular networks and then launch pluripotent regulatory networks remains unknown^{15, 16}.

The mechanisms of miRNA-only reprogramming

As described above, numerous miR-290/302 seed family targets have been identified, but these molecular networks have only been shown in the ES cell context or with forced expression of reprogramming factors OSKM. Previous studies showed that the miR-302 cluster reactivates Oct4 and Nanog through releasing epigenetic repression on the promoter regions by targeting lysine-specific histone demethylases 1 and 2 (AOF1/2), which regulate histone lysine 3 methylation level, and also by targeting methyl-CpG binding proteins 1 and 2 (MECP1/2), which coordinate with DNMT1-mediated gene regulations¹³. However, Subramanyam et al. showed that only Tgfb2r was detectably changed under the same miR-only reprogramming conditions³⁶. A change in expression of MECP2 and other known targets of miR-302 could only be detected in the presence of three or four reprogramming factors³⁶, suggesting that the reprogramming effect of miR-302 is due to modulating regulatory networks in a cell context-dependent manner. Therefore, the miR-302 cluster may exert its reprogramming potential through distinct routes from those of Yamanaka's factors, but can induce reprogrammed cells to eventually reach a similar, if not identical, stage of pluripotency.

Can miRNAs bring us toward the next level of regenerative medicine?

Creating iPS cells by using miRNAs represent a great advance in stem cell biology and regenerative medicine. Inspired by induced reprogramming technology, numerous laboratories have shown that forced expression of specific transcription factors can also transform one cell type to the desired one¹²⁹⁻¹³². In near future, we may be able to alter the

cell identities with high efficiency by transiently introduction of tissue-specific miRNAs only. Since miRNAs can be easily introduced into a variety of cell types by transfection similar to siRNAs, using only mature miRNAs without viral integration can significantly reduce the technical requirement. Oncogene introduction and genomic integration of transgenes can also be eliminated, because transfected miRNAs are transiently expressed in cells with specific half-lives. With efficiency of two orders of magnitude increase relative to OSKM-induced reprogramming¹⁵, utilizing miRNAs provides the best way to date to induce pluripotent stem cells. Despite the great advance of reprogramming technologies, we are only about to understand the molecular basis underneath the reprogramming process. It could be challenging to decipher the molecular regulations in miRNA-mediated reprogramming because miRNAs usually target a large number of genes to modulate biological networks. New high throughput RNA capturing and sequencing technologies may assist in identifying miRNA targets and their roles in reprogramming^{133, 134}.

Given the reprogramming process takes place in a stochastic manner, it is likely that only part of modulating forces from each reprogramming factor affect positively toward inducing pluripotency. Upon introduction of four reprogramming factors into somatic cells, transcriptional networks are disturbed by forced-expressed transcription factors. On the other hands, those factors bind and regulate hundreds of targets to rebuild distinct networks. The types of reconstructed transcriptional networks are possibly decided by what combinations are from the contribution of each reprogramming factor. Since pluripotency is the only expected phenotype, most of the reprogrammed cell types fail and only a few cells working a way out of this chaos to reach the end point, bearing ES-like transcriptome. And since it is a random event upon induced reprogramming, it is unlikely to only have a single and well-defined molecular transition under the coordination of four reprogramming factors, which may vary cell to cell. Secondary tet-inducible reprogramming system may serve this purpose better to shed light on the transcriptome transitions, because the defined dosage of reprogramming factors¹³⁵ may reduce the noise compared with random combinations of OSKM. miRNAs may serve as another tools to decipher the cloud of reprogramming process, since the targets of miRNAs are directed by seed region sequences. However, the progression of miR-induced reprogramming is unknown and the molecular transitions are likely distinct from the ones induced with OSKM, while critical molecular signatures may still conserve. Furthermore, extensive examinations are required to establish standard protocols to create miR-induced pluripotent stem cells, since several discrepancies on miRNA combinations, administering methods, and reprogramming efficiency need to be clarified. Therefore, step-wise dissection of miR-induced reprogramming is required to elucidate the critical features, which are involved in both transcriptional factor-induced and miRNA-induced reprogramming.

Acknowledgments

We are grateful to members of the Rana laboratory for helpful discussions. This work was supported in part by grants from the National Institutes of Health to T.M.R.

BIOGRAPHICAL INFORMATION

Chao-Shun Yang received his Master degree in Department of Biochemistry in 2000 from National Cheng Kung University, Taiwan. He is currently a Ph.D student at University of Massachusetts Medical School and is completing his research dissertation in Dr.Tariq Rana's laboratory at Sanford-Burnham Medical Research Institute, La Jolla, California.

Tariq M. Rana received his Ph.D from the University of California at Davis and was an American Cancer Society fellow at the University of California at Berkeley. He was a

Professor of Biochemistry and Molecular Pharmacology and Director of Program in Chemical Biology at the University of Massachusetts Medical School before joining the Sanford-Burnham Medical Research Institute in 2008. He is currently Professor and Director of the RNA Biology Program at Sanford-Burnham Medical Research Institute, where his laboratory studies RNA regulation of development and disease.

References

1. Takahashi K, Yamanaka S. *Cell*. 2006; 126:663–676. [PubMed: 16904174]
2. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin, Thomson JA. *Science*. 2007; 318:1917–1920. [PubMed: 18029452]
3. Wernig M, Meissner A, Foreman R, Brambrink T, Ku M, Hochedlinger K, Bernstein BE, Jaenisch R. *Nature*. 2007; 448:318–324. [PubMed: 17554336]
4. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. *Cell*. 2007; 131:861–872. [PubMed: 18035408]
5. Meissner A, Wernig M, Jaenisch R. *Nat Biotechnol*. 2007; 25:1177–1181. [PubMed: 17724450]
6. Park IH, Zhao R, West JA, Yabuuchi A, Huo H, Ince TA, Lerou PH, Lensch MW, Daley GQ. *Nature*. 2008; 451:141–146. [PubMed: 18157115]
7. Yamanaka S. *Cell Stem Cell*. 2007; 1:39–49. [PubMed: 18371333]
8. Stadtfeld M, Hochedlinger K. *Genes & development*. 2010; 24:2239–2263. [PubMed: 20952534]
9. Plath K, Lowry WE. *Nature reviews. Genetics*. 2011; 12:253–265. [PubMed: 21415849]
10. Ho R, Chronis C, Plath K. *Journal of cellular physiology*. 2011; 226:868–878. [PubMed: 20945378]
11. Hochedlinger K, Plath K. *Development*. 2009; 136:509–523. [PubMed: 19168672]
12. Warren L, Manos PD, Ahfeldt T, Loh YH, Li H, Lau F, Ebina W, Mandal PK, Smith ZD, Meissner A, Daley GQ, Brack AS, Collins JJ, Cowan C, Schlaeger TM, Rossi DJ. *Cell Stem Cell*. 2010; 7:618–630. [PubMed: 20888316]
13. Lin SL, Chang DC, Lin CH, Ying SY, Leu D, Wu DT. *Nucleic Acids Res*. 2011; 39:1054–1065. [PubMed: 20870751]
14. Lin SL, Chang DC, Chang-Lin S, Lin CH, Wu DT, Chen DT, Ying SY. *Rna*. 2008; 14:2115–2124. [PubMed: 18755840]
15. Anokye-Danso F, Trivedi CM, Jühr D, Gupta M, Cui Z, Tian Y, Zhang Y, Yang W, Gruber PJ, Epstein JA, Morrissey EE. *Cell Stem Cell*. 2011; 8:376–388. [PubMed: 21474102]
16. Miyoshi N, Ishii H, Nagano H, Haraguchi N, Dewi DL, Kano Y, Nishikawa S, Tanemura M, Mimori K, Tanaka F, Saito T, Nishimura J, Takemasa I, Mizushima T, Ikeda M, Yamamoto H, Sekimoto M, Doki Y, Mori M. *Cell Stem Cell*. 2011; 8:633–638. [PubMed: 21620789]
17. Stadtfeld M, Maherali N, Breault DT, Hochedlinger K. *Cell Stem Cell*. 2008; 2:230–240. [PubMed: 18371448]
18. Brambrink T, Foreman R, Welstead GG, Lengner CJ, Wernig M, Suh H, Jaenisch R. *Cell Stem Cell*. 2008; 2:151–159. [PubMed: 18371436]
19. Sridharan R, Tchieu J, Mason MJ, Yachechko R, Kuoy E, Horvath S, Zhou Q, Plath K. *Cell*. 2009; 136:364–377. [PubMed: 19167336]
20. Yang CS, Lopez CG, Rana TM. *Stem Cells*. 2011; 29:1528–1536. [PubMed: 21898684]
21. Hong H, Takahashi K, Ichisaka T, Aoi T, Kanagawa O, Nakagawa M, Okita K, Yamanaka S. *Nature*. 2009; 460:1132–1135. [PubMed: 19668191]
22. Li H, Collado M, Villasante A, Strati K, Ortega S, Canamero M, Blasco MA, Serrano M. *Nature*. 2009; 460:1136–1139. [PubMed: 19668188]
23. Marion RM, Strati K, Li H, Murga M, Blanco R, Ortega S, Fernandez-Capetillo O, Serrano M, Blasco MA. *Nature*. 2009; 460:1149–1153. [PubMed: 19668189]
24. Kawamura T, Suzuki J, Wang YV, Menendez S, Morera LB, Raya A, Wahl GM, Izpisua Belmonte JC. *Nature*. 2009; 460:1140–1144. [PubMed: 19668186]

25. Banito A, Rashid ST, Acosta JC, Li S, Pereira CF, Geti I, Pinho S, Silva JC, Azuara V, Walsh M, Vallier L, Gil J. *Genes & development*. 2009; 23:2134–2139. [PubMed: 19696146]
26. Utikal J, Polo JM, Stadtfeld M, Maherali N, Kulalert W, Walsh RM, Khalil A, Rheinwald JG, Hochedlinger K. *Nature*. 2009; 460:1145–1148. [PubMed: 19668190]
27. Ichida JK, Blanchard J, Lam K, Son EY, Chung JE, Egli D, Loh KM, Carter AC, Di Giorgio FP, Koszka K, Huangfu D, Akutsu H, Liu DR, Rubin LL, Eggan K. *Cell Stem Cell*. 2009; 5:491–503. [PubMed: 19818703]
28. Maherali N, Hochedlinger K. *Curr Biol*. 2009; 19:1718–1723. [PubMed: 19765992]
29. Yang CS, Li Z, Rana TM. *Rna*. 2011; 17:1451–1460. [PubMed: 21693621]
30. Lipchina I, Elkabetz Y, Hafner M, Sheridan R, Mihailovic A, Tuschl T, Sander C, Studer L, Betel D. *Genes & development*. 2011; 25:2173–2186. [PubMed: 22012620]
31. Liao B, Bao X, Liu L, Feng S, Zovoilis A, Liu W, Xue Y, Cai J, Guo X, Qin B, Zhang R, Wu J, Lai L, Teng M, Niu L, Zhang B, Esteban MA, Pei D. *J Biol Chem*. 2011; 286:17359–17364. [PubMed: 21454525]
32. Melton C, Judson RL, Bluelloch R. *Nature*. 2010; 463:621–626. [PubMed: 20054295]
33. Lin SL, Chang DC, Ying SY, Leu D, Wu DT. *Cancer Res*. 2010; 70:9473–9482. [PubMed: 21062975]
34. Li Z, Yang CS, Nakashima K, Rana TM. *Embo J*. 2011; 30:823–834. [PubMed: 21285944]
35. Pfaff N, Fiedler J, Holzmann A, Schambach A, Moritz T, Cantz T, Thum T. *EMBO Rep*. 2011; 12:1153–1159. [PubMed: 21941297]
36. Subramanyam D, Lamouille S, Judson RL, Liu JY, Bucay N, Derynck R, Bluelloch R. *Nature biotechnology*. 2011; 29:443–448.
37. Judson RL, Babiarz JE, Venere M, Bluelloch R. *Nature biotechnology*. 2009; 27:459–461.
38. Cao H, Yang CS, Rana TM. *PLoS One*. 2008; 3:e2820. [PubMed: 18665260]
39. Rana TM. *Nat Rev Mol Cell Biol*. 2007; 8:23–36. [PubMed: 17183358]
40. Li Z, Rana TM. *Acc Chem Res*. 2012; 45:1122–1131. [PubMed: 22304792]
41. Ambros V. *Curr Opin Genet Dev*. 2011; 21:511–517. [PubMed: 21530229]
42. Tiscornia G, Izpisua Belmonte JC. *Genes & development*. 2010; 24:2732–2741. [PubMed: 21159814]
43. Subramanyam D, Bluelloch R. *Curr Opin Genet Dev*. 2011; 21:498–503. [PubMed: 21636265]
44. Kim M, Kasinski AL, Slack FJ. *Lancet Oncol*. 2011; 12:319–321. [PubMed: 21463831]
45. Kasinski AL, Slack FJ. *Curr Opin Mol Ther*. 2010; 12:147–157. [PubMed: 20373258]
46. Esteller M. *Nature reviews. Genetics*. 2011; 12:861–874. [PubMed: 22094949]
47. Farazi TA, Spitzer JJ, Morozov P, Tuschl T. *J Pathol*. 2011; 223:102–115. [PubMed: 21125669]
48. van Kouwenhove M, Kedde M, Agami R. *Nat Rev Cancer*. 2011; 11:644–656. [PubMed: 21822212]
49. Chen J, Wang G, Lu C, Guo X, Hong W, Kang J, Wang J. *PLoS One*. 2012; 7:e40849. [PubMed: 22808276]
50. Hanna JH, Saha K, Jaenisch R. *Cell*. 2010; 143:508–525. [PubMed: 21074044]
51. Orkin SH, Hochedlinger K. *Cell*. 2011; 145:835–850. [PubMed: 21663790]
52. Lai MI, Wendy-Yeo WY, Ramasamy R, Nordin N, Rosli R, Veerakumarasivam A, Abdullah S. *J Assist Reprod Genet*. 2011; 28:291–301. [PubMed: 21384252]
53. Han JW, Yoon YS. *Antioxid Redox Signal*. 2012; 17:205–223. [PubMed: 22044221]
54. Gonzalez F, Boue S, Izpisua Belmonte JC. *Nature reviews. Genetics*. 2011; 12:231–242. [PubMed: 21339765]
55. Tiscornia G, Vivas EL, Izpisua Belmonte JC. *Nat Med*. 2011; 17:1570–1576. [PubMed: 22146428]
56. Robinton DA, Daley GQ. *Nature*. 2012; 481:295–305. [PubMed: 22258608]
57. Grskovic M, Javaherian A, Strulovici B, Daley GQ. *Nat Rev Drug Discov*. 2011; 10:915–929. [PubMed: 22076509]
58. Lipchina I, Studer L, Betel D. *Cell Cycle*. 2012; 11:1517–1523. [PubMed: 22436490]
59. Mallanna SK, Rizzino A. *Dev Biol*. 2010; 344:16–25. [PubMed: 20478297]

60. Kuo CH, Deng JH, Deng Q, Ying SY. Biochemical and biophysical research communications. 2012; 417:11–16. [PubMed: 22138244]
61. Wang Y, Blelloch R. Results Probl Cell Differ. 2011; 53:459–472. [PubMed: 21630156]
62. Heinrich EM, Dimmeler S. Circulation research. 2012; 110:1014–1022. [PubMed: 22461365]
63. Li MA, He L. Bioessays. 2012; 34:670–680. [PubMed: 22674461]
64. Pfaff N, Moritz T, Thum T, Cantz T. J Mol Med (Berl). 2012; 90:747–752. [PubMed: 22684238]
65. Yamanaka S. Nature. 2009; 460:49–52. [PubMed: 19571877]
66. Hanna J, Saha K, Pando B, van Zon J, Lengner CJ, Creighton MP, van Oudenaarden A, Jaenisch R. Nature. 2009; 462:595–601. [PubMed: 19898493]
67. MacArthur BD, Please CP, Oreffo RO. PLoS One. 2008; 3:e3086. [PubMed: 18769478]
68. Koche RP, Smith ZD, Adli M, Gu H, Ku M, Gnirke A, Bernstein BE, Meissner A. Cell Stem Cell. 2011; 8:96–105. [PubMed: 21211784]
69. Li R, Liang J, Ni S, Zhou T, Qing X, Li H, He W, Chen J, Li F, Zhuang Q, Qin B, Xu J, Li W, Yang J, Gan Y, Qin D, Feng S, Song H, Yang D, Zhang B, Zeng L, Lai L, Esteban MA, Pei D. Cell Stem Cell. 2010; 7:51–63. [PubMed: 20621050]
70. Samavarchi-Tehrani P, Golipour A, David L, Sung HK, Beyer TA, Datti A, Woltjen K, Nagy A, Wrana JL. Cell Stem Cell. 2010; 7:64–77. [PubMed: 20621051]
71. Wanzel M, Herold S, Eilers M. Trends Cell Biol. 2003; 13:146–150. [PubMed: 12628347]
72. Chang TC, Yu D, Lee YS, Wentzel EA, Arking DE, West KM, Dang CV, Thomas-Tikhonenko A, Mendell JT. Nat Genet. 2008; 40:43–50. [PubMed: 18066065]
73. Lotterman CD, Kent OA, Mendell JT. Cell Cycle. 2008; 7:2493–2499. [PubMed: 18719378]
74. Gao P, Tchernyshyov I, Chang TC, Lee YS, Kita K, Ochi T, Zeller KI, De Marzo AM, Van Eyk JE, Mendell JT, Dang CV. Nature. 2009; 458:762–765. [PubMed: 19219026]
75. Chang TC, Zeitels LR, Hwang HW, Chivukula RR, Wentzel EA, Dews M, Jung J, Gao P, Dang CV, Beer MA, Thomas-Tikhonenko A, Mendell JT. Proceedings of the National Academy of Sciences of the United States of America. 2009; 106:3384–3389. [PubMed: 19211792]
76. Lin CH, Lin C, Tanaka H, Fero ML, Eisenman RN. PLoS One. 2009; 4:e7839. [PubMed: 19915707]
77. Lin CH, Jackson AL, Guo J, Linsley PS, Eisenman RN. Embo J. 2009; 28:3157–3170. [PubMed: 19745813]
78. Smith K, Dalton S. Regen Med. 2010; 5:947–959. [PubMed: 21082893]
79. Smith KN, Singh AM, Dalton S. Cell Stem Cell. 2010; 7:343–354. [PubMed: 20804970]
80. Mott JL, Kurita S, Cazanave SC, Bronk SF, Werneburg NW, Fernandez-Zapico ME. J Cell Biochem. 2010; 110:1155–1164. [PubMed: 20564213]
81. Liu G, Friggeri A, Yang Y, Milosevic J, Ding Q, Thannickal VJ, Kaminski N, Abraham E. The Journal of experimental medicine. 2010; 207:1589–1597. [PubMed: 20643828]
82. Thum T, Gross C, Fiedler J, Fischer T, Kissler S, Bussen M, Galuppo P, Just S, Rottbauer W, Frantz S, Castoldi M, Soutschek J, Kotliansky V, Rosenwald A, Basson MA, Licht JD, Pena JT, Rouhanifard SH, Muckenthaler MU, Tuschl T, Martin GR, Bauersachs J, Engelhardt S. Nature. 2008; 456:980–984. [PubMed: 19043405]
83. Yang J, van Oosten AL, Theunissen TW, Guo G, Silva JC, Smith A. Cell Stem Cell. 2010; 7:319–328. [PubMed: 20804969]
84. Wray J, Kalkan T, Smith AG. Biochem Soc Trans. 2010; 38:1027–1032. [PubMed: 20658998]
85. Nichols J, Silva J, Roode M, Smith A. Development. 2009; 136:3215–3222. [PubMed: 19710168]
86. Ying QL, Wray J, Nichols J, Battle-Morera L, Doble B, Woodgett J, Cohen P, Smith A. Nature. 2008; 453:519–523. [PubMed: 18497825]
87. Park SY, Lee JH, Ha M, Nam JW, Kim VN. Nat Struct Mol Biol. 2009; 16:23–29. [PubMed: 19079265]
88. Kim HH, Kuwano Y, Srikantan S, Lee EK, Martindale JL, Gorospe M. Genes & development. 2009; 23:1743–1748. [PubMed: 19574298]
89. Rybak A, Fuchs H, Smirnova L, Brandt C, Pohl EE, Nitsch R, Wulczyn FG. Nat Cell Biol. 2008; 10:987–993. [PubMed: 18604195]

90. Sampson VB, Rong NH, Han J, Yang Q, Aris V, Soteropoulos P, Petrelli NJ, Dunn SP, Krueger LJ. *Cancer Res.* 2007; 67:9762–9770. [PubMed: 17942906]
91. Park SM, Shell S, Radjabi AR, Schickel R, Feig C, Boyerinas B, Dinulescu DM, Lengyel E, Peter ME. *Cell Cycle.* 2007; 6:2585–2590. [PubMed: 17957144]
92. He L, He X, Lim LP, de Stanchina E, Xuan Z, Liang Y, Xue W, Zender L, Magnus J, Ridzon D, Jackson AL, Linsley PS, Chen C, Lowe SW, Cleary MA, Hannon GJ. *Nature.* 2007; 447:1130–1134. [PubMed: 17554337]
93. Raver-Shapira N, Marciano E, Meiri E, Spector Y, Rosenfeld N, Moskovits N, Bentwich Z, Oren M. *Mol Cell.* 2007; 26:731–743. [PubMed: 17540598]
94. Chang TC, Wentzel EA, Kent OA, Ramachandran K, Mullendore M, Lee KH, Feldmann G, Yamakuchi M, Ferlito M, Lowenstein CJ, Arking DE, Beer MA, Maitra A, Mendell JT. *Mol Cell.* 2007; 26:745–752. [PubMed: 17540599]
95. Choi YJ, Lin CP, Ho JJ, He X, Okada N, Bu P, Zhong Y, Kim SY, Bennett MJ, Chen C, Ozturk A, Hicks GG, Hannon GJ, He L. *Nat Cell Biol.* 2011; 13:1353–1360. [PubMed: 22020437]
96. Jain AK, Allton K, Iacovino M, Mahen E, Milczarek RJ, Zwaka TP, Kyba M, Barton MC. *PLoS Biol.* 2012; 10:e1001268. [PubMed: 22389628]
97. Siemens H, Jackstadt R, Hunten S, Kaller M, Menssen A, Gotz U, Hermeking H. *Cell Cycle.* 2011; 10:4256–4271. [PubMed: 22134354]
98. Kim NH, Kim HS, Li XY, Lee I, Choi HS, Kang SE, Cha SY, Ryu JK, Yoon D, Fearon ER, Rowe RG, Lee S, Maher CA, Weiss SJ, Yook JI. *J Cell Biol.* 2011; 195:417–433. [PubMed: 22024162]
99. Xu N, Papagiannakopoulos T, Pan G, Thomson JA, Kosik KS. *Cell.* 2009; 137:647–658. [PubMed: 19409607]
100. Liu T, Cheng W, Huang Y, Huang Q, Jiang L, Guo L. *Exp Cell Res.* 2012; 318:424–434. [PubMed: 22200372]
101. Ma L, Teruya-Feldstein J, Weinberg RA. *Nature.* 2007; 449:682–688. [PubMed: 17898713]
102. Kong W, Yang H, He L, Zhao JJ, Coppola D, Dalton WS, Cheng JQ. *Mol Cell Biol.* 2008; 28:6773–6784. [PubMed: 18794355]
103. Gregory PA, Bert AG, Paterson EL, Barry SC, Tsykin A, Farshid G, Vadas MA, Khew-Goodall Y, Goodall GJ. *Nat Cell Biol.* 2008; 10:593–601. [PubMed: 18376396]
104. Wiklund ED, Catts VS, Catts SV, Ng TF, Whitaker NJ, Brown AJ, Lutze-Mann LH. *Int J Cancer.* 2010; 126:28–40. [PubMed: 19662652]
105. Burk U, Schubert J, Wellner U, Schmalhofer O, Vincan E, Spaderna S, Brabletz T. *EMBO Rep.* 2008; 9:582–589. [PubMed: 18483486]
106. Wellner U, Schubert J, Burk UC, Schmalhofer O, Zhu F, Sonntag A, Waldvogel B, Vannier C, Darling D, zur Hausen A, Brunton VG, Morton J, Sansom O, Schuler J, Stemmler MP, Herzberger C, Hopt U, Keck T, Brabletz S, Brabletz T. *Nat Cell Biol.* 2009; 11:1487–1495. [PubMed: 19935649]
107. Chen J, Wang L, Matyunina LV, Hill CG, McDonald JF. *Gynecol Oncol.* 2011; 121:200–205. [PubMed: 21277012]
108. Ma L, Reinhardt F, Pan E, Soutschek J, Bhat B, Marcusson EG, Teruya-Feldstein J, Bell GW, Weinberg RA. *Nature biotechnology.* 2010; 28:341–347.
109. Wang HR, Zhang Y, Ozdamar B, Ogunjimi AA, Alexandrova E, Thomsen GH, Wrana JL. *Science.* 2003; 302:1775–1779. [PubMed: 14657501]
110. Xiang X, Zhuang X, Ju S, Zhang S, Jiang H, Mu J, Zhang L, Miller D, Grizzle W, Zhang HG. *Oncogene.* 2011; 30:3440–3453. [PubMed: 21460854]
111. Gregory PA, Bracken CP, Bert AG, Goodall GJ. *Cell Cycle.* 2008; 7:3112–3118. [PubMed: 18927505]
112. Wiklund ED, Kjems J, Clark SJ. *Epigenomics.* 2010; 2:823–840. [PubMed: 22122085]
113. Park SM, Gaur AB, Lengyel E, Peter ME. *Genes & development.* 2008; 22:894–907. [PubMed: 18381893]
114. Chen J, Liu J, Yang J, Chen Y, Ni S, Song H, Zeng L, Ding K, Pei D. *Cell research.* 2011; 21:205–212. [PubMed: 21135873]

115. Wang Y, Baskerville S, Shenoy A, Babiarz JE, Baehner L, Blelloch R. *Nat Genet.* 2008; 40:1478–1483. [PubMed: 18978791]
116. Kanellopoulou C, Muljo SA, Kung AL, Ganesan S, Drapkin R, Jenuwein T, Livingston DM, Rajewsky K. *Genes & development.* 2005; 19:489–501. [PubMed: 15713842]
117. Murchison EP, Partridge JF, Tam OH, Cheloufi S, Hannon GJ. *Proceedings of the National Academy of Sciences of the United States of America.* 2005; 102:12135–12140. [PubMed: 16099834]
118. Houbaviy HB, Murray MF, A. Sharp P. *Dev Cell.* 2003; 5:351–358. [PubMed: 12919684]
119. Suh MR, Lee Y, Kim JY, Kim SK, Moon SH, Lee JY, Cha KY, Chung HM, Yoon HS, Moon SY, Kim VN, Kim KS. *Dev Biol.* 2004; 270:488–498. [PubMed: 15183728]
120. Landgraf P, Rusu M, Sheridan R, Sewer A, Iovino N, Aravin A, Pfeffer S, Rice A, Kamphorst AO, Landthaler M, Lin C, Socci ND, Hermida L, Fulci V, Chiaretti S, Foa R, Schliwka J, Fuchs U, Novosel A, Muller RU, Schermer B, Bissels U, Inman J, Phan Q, Chien M, Weir DB, Choksi R, De Vita G, Frezzetti D, Trompeter HI, Hornung V, Teng G, Hartmann G, Palkovits M, Di Lauro R, Wernet P, Macino G, Rogler CE, Nagle JW, Ju J, Papavasiliou FN, Benzing T, Lichter P, Tam W, Brownstein MJ, Bosio A, Borkhardt A, Russo JJ, Sander C, Zavolan M, Tuschl T. *Cell.* 2007; 129:1401–1414. [PubMed: 17604727]
121. Marson A, Levine SS, Cole MF, Frampton GM, Brambrink T, Johnstone S, Guenther MG, Johnston WK, Wernig M, Newman J, Calabrese JM, Dennis LM, Volkert TL, Gupta S, Love J, Hannett N, Sharp PA, Bartel DP, Jaenisch R, Young RA. *Cell.* 2008; 134:521–533. [PubMed: 18692474]
122. Card DA, Hebbard PB, Li L, Trotter KW, Komatsu Y, Mishina Y, Archer TK. *Mol Cell Biol.* 2008; 28:6426–6438. [PubMed: 18710938]
123. Rosa A, Spagnoli FM, Brivanlou AH. *Dev Cell.* 2009; 16:517–527. [PubMed: 19386261]
124. Benetti R, Gonzalo S, Jaco I, Munoz P, Gonzalez S, Schoeftner S, Murchison E, Andl T, Chen T, Klatt P, Li E, Serrano M, Millar S, Hannon G, Blasco MA. *Nat Struct Mol Biol.* 2008; 15:268–279. [PubMed: 18311151]
125. Sinkkonen L, Hugenschmidt T, Berninger P, Gaidatzis D, Mohn F, Artus-Revel CG, Zavolan M, Svoboda P, Filipowicz W. *Nat Struct Mol Biol.* 2008; 15:259–267. [PubMed: 18311153]
126. Kamata M, Liang M, Liu S, Nagaoka Y, Chen IS. *PLoS One.* 2010; 5:e11834. [PubMed: 20676373]
127. Wang T, Chen K, Zeng X, Yang J, Wu Y, Shi X, Qin B, Zeng L, Esteban MA, Pan G, Pei D. *Cell Stem Cell.* 2011; 9:575–587. [PubMed: 22100412]
128. Luningschror P, Stocker B, Kaltschmidt B, Kaltschmidt C. *Stem Cells.* 2012; 30:655–664. [PubMed: 22232084]
129. Ambasudhan R, Talantova M, Coleman R, Yuan X, Zhu S, Lipton SA, Ding S. *Cell Stem Cell.* 2011; 9:113–118. [PubMed: 21802386]
130. Yoo AS, Sun AX, Li L, Shcheglovitov A, Portmann T, Li Y, Lee-Messer C, Dolmetsch RE, Tsien RW, Crabtree GR. *Nature.* 2011; 476:228–231. [PubMed: 21753754]
131. Jopling C, Boue S, Izpisua Belmonte JC. *Nat Rev Mol Cell Biol.* 2011; 12:79–89. [PubMed: 21252997]
132. Chambers SM, Studer L. *Cell.* 2011; 145:827–830. [PubMed: 21663788]
133. Baigude H, Ahsanullah, Li Z, Zhou Y, Rana TM. *Angew Chem Int Ed Engl.* 2012; 51:5880–5883. [PubMed: 22566243]
134. Zhou H, Arcila ML, Li Z, Lee EJ, Henzler C, Liu J, Rana TM, Kosik KS. *Nucleic Acids Res.* 2012; 40:5864–5875. [PubMed: 22434881]
135. Markoulaki S, Hanna J, Beard C, Carey BW, Cheng AW, Lengner CJ, Dausman JA, Fu D, Gao Q, Wu S, Cassady JP, Jaenisch R. *Nature biotechnology.* 2009; 27:169–171.
136. Wilson KD, Venkatasubrahmanyam S, Jia F, Sun N, Butte AJ, Wu JC. *Stem cells and development.* 2009; 18:749–758. [PubMed: 19284351]

Figure 1. MicroRNAs play important roles to support reprogramming progress

A. MicroRNAs in the initial step of reprogramming.

c-Myc has been shown to disrupt the molecular networks of somatic cells to promote the initiation of reprogramming. c-Myc is also the main regulator of miRNA expression early in reprogramming with miR-21 and miR-29a being the main targets of c-Myc at this stage. Let-7 is repressed by c-Myc through Lin-28b-mediated regulation, but this is not detected during early reprogramming. c-Myc also induces the oncogenic stress response by activating p53/miR-34.

B. MicroRNAs in mesenchymal-to-epithelial transition (MET) of reprogramming.

miR-10b and miR-155 promote mesenchymal-like properties, while miR-205 and the miR-200 family (miR-429) promote epithelial-like characteristics. c-Myc coordinates with Klf4, to induce miR-429 upon reprogramming, which accelerates the transition step. miR-290/302 seed family, miR-302 cluster and miR-106b cluster, have been shown to enhance epithelial properties.

C. MicroRNAs in late stage of reprogramming.

In this last step of iPSC maturation, the ES-specific molecular signatures are reactivated, including ES-specific miRNAs, miR-290, and miR-302 clusters. c-Myc plays major roles in inducing miR-290 and miR-302 clusters. miR-290, miR-302, and other miR-290/302 seed family miRNAs also play important roles to restore the ESC properties.

Table 1Enriched miRNAs in human ES and/or iPS cells relative to IMR90 fibroblasts¹³⁶

Both in ES and iPS cells		Only in ES cells
hsa-miR-17	hsa-miR-205	hsa-miR-96
hsa-miR-18a	hsa-miR-302a	hsa-miR-222*
hsa-miR-18b	hsa-miR-302a*	hsa-miR-371-5p
hsa-miR-19a	hsa-miR-302b	hsa-miR-372
hsa-miR-19b	hsa-miR-302b*	hsa-miR-373
hsa-miR-20a	hsa-miR-302c	hsa-miR-512-3p
hsa-miR-20b	hsa-miR-302c*	hsa-miR-515-5p
hsa-miR-20b*	hsa-miR-302d	hsa-miR-516a-5p
hsa-miR-25	hsa-miR-302d*	hsa-miR-516b
hsa-miR-30e	hsa-miR-363	hsa-miR-518b
hsa-miR-92a	hsa-miR-363*	hsa-miR-518d-5p
hsa-miR-92b	hsa-miR-421	hsa-miR-518e*
hsa-miR-93	hsa-miR-486-5p	hsa-miR-518f*
hsa-miR-106a	hsa-miR-489	hsa-miR-519c-3p
hsa-miR-106b	hsa-miR-498	hsa-miR-520f
hsa-miR-140a	hsa-miR-517a	hsa-miR-520g
hsa-miR-182	hsa-miR-517b	hsa-miR-520h
hsa-miR-183	hsa-miR-638	hsa-miR-525-5p
hsa-miR-187	hsa-miR-663	hsa-miR-629
hsa-miR-200c	hsa-miR-923	

Table 2

miR-290/302 seed region family

miRNAs	5'-3' guide strand sequence[#]
miR-290 cluster	
mmu-miR-290-3p	aaagugccgcuaguuuaagccc
mmu-miR-291a-3p	aaagugcuuccacuugugugc
mmu-miR-292-3p	aaagugccgagguuuugagugu
mmu-miR-291b-3p	aaagugcauccauuuuguuugu
mmu-miR-294-3p	aaagugcuuccuuuuugugugu
mmu-miR-295-3p	aaagugcuacuauuuugagucu
miR-302b cluster	
mmu-miR-302b-3p	uaagugcuuccauguuuaguag
mmu-miR-302c-3p	aaagugcuuccauguuucagugg
mmu-miR-302a-3p	uaagugcuuccauguuuugguga
mmu-miR-302d-3p	uaagugcuuccauguuugagugu
miR-17 cluster	
mmu-miR-17-5p	caaagugcuuacagugcagguag
mmu-miR-20a-5p	uaaagugcuuauagugcagguag
miR-106b cluster	
mmu-miR-106b-5p	uaaagugcugacagugcagau
mmu-miR-93-5p	caaagugcuuucugugcagguag
miR-106a cluster	
mmu-miR-106a	caaagugcuaacagugcagguag
mmu-miR-20b	caaagugcucauagugcagguag
miR-130/301/721 family	
mmu-miR-130a	cagugcaauguuuaaagggcgau
mmu-miR-130b	cagugcaaugaugaagagggcgau
mmu-miR-301a	cagugcaauaguauugucaaaagc
mmu-miR-301b	cagugcaaugguauugucaaaagc
mmu-miR-721	cagugcaauuaaagggggaa

[#] Bold and enlarge nucleotides represent conserved regions