

Transcription factor and microRNA co-regulatory loops: important regulatory motifs in biological processes and diseases

Hong-Mei Zhang, Shuzhen Kuang, Xushen Xiong, Tianliuyun Gao, Chenglin Liu and An-Yuan Guo

Submitted: 10th September 2013; Received (in revised form): 16th October 2013

Abstract

Transcription factors (TFs) and microRNAs (miRNAs) can jointly regulate target gene expression in the forms of feed-forward loops (FFLs) or feedback loops (FBLs). These regulatory loops serve as important motifs in gene regulatory networks and play critical roles in multiple biological processes and different diseases. Major progress has been made in bioinformatics and experimental study for the TF and miRNA co-regulation in recent years. To further speed up its identification and functional study, it is indispensable to make a comprehensive review. In this article, we summarize the types of FFLs and FBLs and their identified methods. Then, we review the behaviors and functions for the experimentally identified loops according to biological processes and diseases. Future improvements and challenges are also discussed, which includes more powerful bioinformatics approaches and high-throughput technologies in TF and miRNA target prediction, and the integration of networks of multiple levels.

Keywords: FFLs; FBLs; TF-miRNA co-regulatory network; target prediction; cell proliferation

INTRODUCTION

Transcription factors (TFs) are modular proteins that regulate gene transcription through binding to the promoter region of target genes by their DNA-binding domain [1]. MicroRNAs (miRNAs) are a kind of small noncoding RNAs ~22 nucleotides in length. They are able to silence the expression of target genes by mRNA degradation or translational inhibition [2]. Both TFs and miRNAs are involved in the regulation of various biological processes, including cell proliferation, differentiation and apoptosis. Thus, the dysregulation of TFs and miRNAs is associated with many diseases.

Gene expression regulation is a complex biological process, which is controlled by various factors including proteins and RNAs in multiple levels. As key regulators of gene expression, TFs and miRNAs are able to co-regulate the expression of targets in forms of feed-forward loops (FFLs) and feedback loops (FBLs) [3,4]. These two kinds of loops are important motifs in gene regulatory networks, which were initially proposed to describe the co-regulation between different TFs on the same target [5,6]. However, TFs and miRNAs can also co-regulate gene expression in the same ways. Several studies reported that the TF-miRNA co-regulation was

Corresponding author. An-Yuan Guo, Department of Biomedical Engineering, College of Life Science and Technology, Huazhong University of Science and Technology (HUST), Wuhan 430074, China. Tel.: +86-27-8779-3177; Fax: +86-27-8779-3177; E-mail: guoay@mail.hust.edu.cn

Hong-Mei Zhang is a Ph.D. candidate in Dr. An-Yuan Guo's group at HUST, working on the construction of TF-miRNA co-regulatory network and the integrated analysis of mRNA and miRNA expression profiles.

Shuzhen Kuang is a master student in Dr. An-Yuan Guo's group. Her researches are focused on the regulatory analysis of miRNAs and genes in human diseases.

Xushen Xiong is an undergraduate student majoring in bioinformatics at HUST.

Tianliuyun Gao is an undergraduate student majoring in bioinformatics at HUST.

Chenglin Liu is an undergraduate student majoring in electronic and information engineering at HUST.

An-Yuan Guo is a Professor in Bioinformatics at HUST, China. His research interests focus on bioinformatics and systems biology in complex diseases, bioinformatics database construction and comparative genomics.

prevalent in animal genomes [3,4,7]. The TF-miRNA FFLs influence many aspects of normal cells and diseases. TF-miRNA FFL has a specific function in noise buffering effect. It can minimize the response to stochastic signaling noise and homeostatically maintain steady-state levels of the target protein by tuning the translation of its target in a direction opposite to that of the signal [8]. This robustness imbued by FFL can protect cells against rapidly flipping between alternative cell decisions in the development process [8]. The TF-miRNA FFL may also facilitate the ‘miRNA-target spatiotemporal avoidance’ phenomenon, in which a miRNA and its targets are expressed in adjacent tissues or in consecutive developmental stages for some cases [9]. FBLs also play critical roles in cell differentiation and development. Inui *et al.* [8] elucidated that double-negative FBLs could generate mutually exclusive or bistable expression of the miRNA and TF, and convert a transient signal into a longer lasting cellular response.

Much progress has been made in the study for TF and miRNA co-regulation in recent years. To further speed up its identification and functional study, it is necessary to summarize the methods and review its influences on cellular processes and diseases. In this review, we summarize the types of FFLs/FBLs and their identified methods, as well as the functions in biological processes and diseases. The future challenges and perspectives on TF-miRNA co-regulation are also discussed.

TYPES OF FFLs AND FBLs

In this part, we briefly describe the types and potential functions of FFLs and FBLs. The FFL is a motif in which a TF regulates a miRNA or a miRNA represses a TF, and both of them co-regulate a joint target. FFLs can be divided into three types according to the master regulator: TF-FFL, miRNA-FFL and composite FFL (Figure 1a) [3]. In a TF-FFL, TF is the master regulator, which regulates its partner miRNA and their mutual target, while in a miRNA-FFL, miRNA is the master regulator. TF-FFL and miRNA-FFL can combine into a composite FFL, in which TF and miRNA regulate each other.

In another way, FFLs also can be classified into coherent and incoherent loops based on the effects of miRNAs and TFs on their mutual targets. In a coherent FFL, the regulatory paths have the same

effects on the target (either activation or repression) (Figure 1b), whereas in an incoherent loop the expression of the target is controlled by two reverse paths (Figure 1c). Thus, coherent and incoherent FFLs could lead to different effects on gene regulation. The left graph in Figure 1b shows a typical coherent loop, in which the miRNA transcription is induced by a TF and they jointly repress the expression of their mutual target. This kind of circuit serves as a surveillance mechanism to suppress the expression of ‘leaky’ genes that should not be expressed in specific cells [4,10]. For example, YAN is repressed by Pnt-p1 and miR-7 in the differentiation of photoreceptors [11]. The Figure 1c shows the types of incoherent FFLs and the left one is a typical FFL that has two advantages on regulating gene expression. Firstly, in this case miRNAs have a fine-tuning function to keep the expression level of targets in correct ranges, and the co-expression of miRNAs with their targets allow miRNAs to better fine-tune the stable status of targets [4,10]. Secondly, it prevents undesired activation of targets from stochastic signaling. Such noise buffering helps to maintain the homeostasis of target proteins and the uniformity in their expression profiles within cell populations [12].

The FBLs are not abundant in pure transcriptional networks [13], whereas they are recurrent between miRNAs and TFs in a variety of organisms [7]. The TF-miRNA FBL is a motif in which TF and miRNA regulate each other, and each of them regulates their targets separately. There are two types of FBLs according to the regulation of TFs on miRNAs: single-negative and double-negative loop (Figure 1d). The double-negative loop, in which a miRNA and a TF repress each other, has two steady states: either TF is on and miRNA is off, or the opposite [14]. This kind of FBL can act as a toggle switch between two different fates and therefore play key roles in cell differentiation [15,16]. For instance, the double-negative FBL composed of miR-200 family and ZEB1/SIP1 controls the epithelial to mesenchymal transition (EMT) [15]. In the single-negative loop, a TF activates the transcription of a miRNA, which in turn inhibits the translation of TF. This kind of FBL allows a tightly control for the protein level of TF. For example, the single-negative FBL consisting of miR-17-92 cluster and TF E2F1 is critical for balancing the protein level of E2F1 and controlling the progress of cell cycle [17,18].

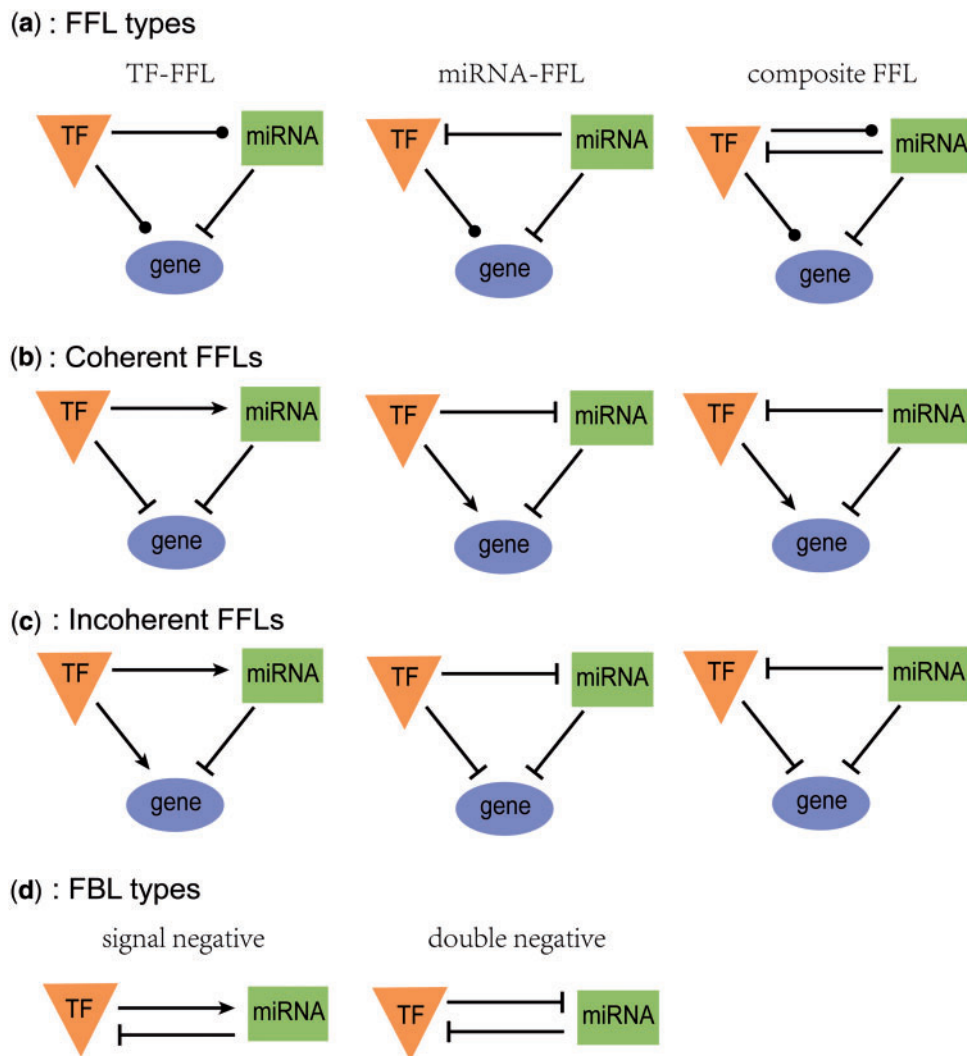


Figure 1: FFL and FBL types. **(a)** Three types of FFLs classified by the master regulator. Blunt arrows with dot end represent transcriptional activation or repression. **(b)** Coherent FFLs. In this kind of FFLs, two paths that regulate target gene have the same effects (either activation or repression). **(c)** Incoherent FFLs. The target gene is regulated by two opposite paths. **(d)** FBL types. Nodes: triangles are TFs; rectangles are miRNAs; ovals are genes; Edges: sharp arrow means activation; T-shaped arrow represents repression.

CONSTRUCTION OF TF-miRNA CO-REGULATORY NETWORKS

As the importance of TF-miRNA FFLs and FBLs was realized, several groups including us employed different strategies to identify these motifs and construct TF-miRNA co-regulatory networks [19,20].

Web servers and databases

Several web servers were developed by other groups to construct TF-miRNA regulatory networks through integrating gene and miRNA expression profiles with target prediction, such as dChip-GemiNI (<http://www.canevolve.org/dChip-GemiNi/>), MAGIA

(<http://gencomp.bio.unipd.it/magia2/>) and mirConnX (<http://www.benoslab.pitt.edu/mirconnx>) [21–23]. The dChip-GemiNI ranks the predicted FFLs by their explanatory power to account for the differential expression of genes and miRNAs between two biological conditions [21]. MAGIA performs an integrative approach to detect TF-FFLs and miRNA-FFLs [22]. MirConnX combines sequence information with gene expression data to identify all kinds of FFLs [23]. Both of MAGIA and mirConnX can accept the control/disease and time-series data, which expands their application range. MIR@NT@N (<http://mir-onton.uni.lu>) is a framework that predicts regulatory networks and subnetworks, including FFLs and FBLs,

based on interactions among TFs, miRNAs and genes obtained from a large-scale database [24]. In addition, several databases were constructed for TF-miRNA co-regulation. For example, CircuitsDB is a database devoted to the study of human and mouse TF-miRNA FFLs by predictions [25]. This database also characterized MYC-specific FFLs through collecting experimentally validated interactions of MYC, miRNA and their joint targets from independent databases [26]. TransmiR curated >700 TF-miRNA regulatory entries in 16 organisms from several hundred publications [27].

Each of these web tools or frameworks has their unique characteristics, but they share many common elements in the process of identifying FFLs and constructing network. According to these methods, we summarized a universal and comprehensive pipeline for constructing the TF-miRNA co-regulatory networks. The key steps are identifying regulatory relationships among TFs, miRNAs and targets.

TF-gene regulation

There are ~2000 TF genes in the human genome [28,29]. However, only partial targets of a small group of TFs have been detected based on the results of individual and high-throughput experiments. Chromatin immunoprecipitation (ChIP) followed by sequencing (ChIP-seq) or by microarray hybridization (ChIP-chip) are two high-throughput technologies to detect TF binding regions in genome-wide and have greatly accelerated the identification of TF targets. However, they also generate a large number of false positives, which can be reduced by integrating the expression profile of TF perturbation [30].

To date, there are several strategies to obtain TF targets by experiments and predictions. (i) We can collect the experimentally verified TF targets from publications or databases such as TRANSFAC [31]. (ii) The huge amount of ChIP-seq and ChIP-chip data generated by ENCODE or other projects are useful resources for TF targets identification. These data are available at NCBI-GEO, UCSC genome browser, CHEA (<http://amp.pharm.mssm.edu/lib/chea.jsp>) [32] and Factorbook.org (<http://factorbook.org/>) [33]. (iii) The TF binding sites (TFBSs) of several hundreds of human TFs have been characterized by experiments and these TFBS matrices are available in the JASPAR and TRANSFAC databases [31,34]. We can predict TF targets through

searching for these TFBS matrices in gene promoters. UCSC genome browser also provides predicted TFBS data across the genome. In practice, we could integrate several of these methods and data to obtain comprehensive and accurate TF targets.

TF-miRNA regulation

Currently, most miRNAs are believed to be transcribed by RNA Polymerase II [35]. Therefore, TFs will bind to the miRNA promoter regions to either activate or repress their transcription. All of those methods for predicting TF target genes can also be applied to predict TF target miRNAs. However, there are some differences on the promoter identification between protein-coding genes and miRNAs. For miRNAs in a cluster, which are transcribed as a single unit [36], it prefers to use the upstream region of the first pre-miRNA as the promoter of the miRNA cluster. In another way, there are intronic and intergenic miRNAs according to their locations relative to protein coding genes. Several studies reported that some intronic miRNAs may be transcribed with their host genes and some are not co-expressed [37–39]. For the intronic miRNAs transcribed with their host genes, the promoters of the host genes should be used. For the intronic miRNAs that transcribe independently and the intergenic miRNAs, the upstream regions of their pre-miRNAs are used as the promoters. Thus, the promoter regions of different kinds of miRNAs should be obtained separately when we plan to predict the TFs regulation on miRNAs.

miRNA-gene/TF regulation

Up to now, there are more than a dozen of miRNA target prediction algorithms, such as TargetScan [40], miRanda [41], PITA [42], Pictar [43] and RNAhybrid [44]. However, different prediction algorithms are not consistent with each other. Thus, users often combine the results of several prediction algorithms to reduce false positives. In addition, some studies combined target prediction algorithms with miRNA and mRNA expression profiles to detect miRNA targets, such as miRNAMap 2.0 [45] and MiRonTop [46]. miRNA-target interactions verified by experiments were also collected by several databases. TarBase [47] and miRTarBase [48] manually curated the experimentally verified miRNA targets for nearly 20 species. MiRecords not only curated the validated targets but also predicted miRNA targets with 11

established prediction programs [49]. MiR2Disease provided a resource of miRNA dysregulation in diseases and collected the verified miRNA targets [50]. In practice, we may combine the experimentally verified miRNA targets with predicted targets by different methods.

Construction of TF-miRNA regulatory networks

According to the definition of FFLs and FBLs in Figure 1, they can be easily extracted based on the predicted and/or validated relationships among TFs, miRNAs and genes. Fisher's exact test and randomization processes are usually used to evaluate whether the FFLs and FBLs are significantly enriched from

genome background. The detail of these two test methods can be found in the previous studies [3,19,51]. These FFLs and FBLs would be merged together to form a comprehensive TF-miRNA regulatory network, which can be visualized by software such as Cytoscape [52]. The workflow for constructing TF-miRNA regulatory networks is shown in Figure 2.

TF-miRNA CO-REGULATION IN CELL PROLIFERATION

Cell proliferation is an important process during the growth and development of individuals, which is regulated by a complex network consisting of

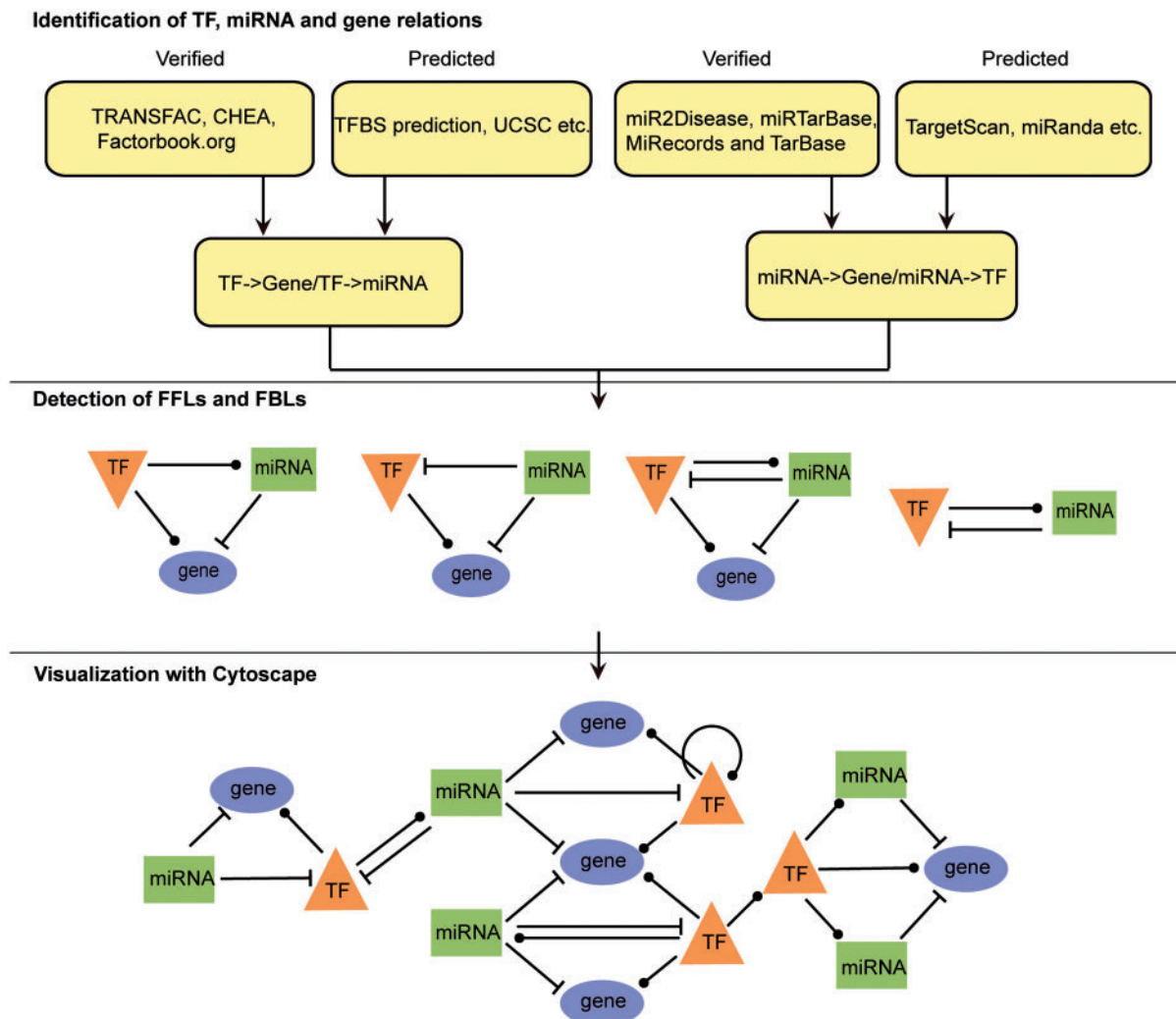


Figure 2: A workflow for constructing the TF-miRNA co-regulatory network. There are three steps in this process. Firstly, obtaining the regulatory relations among TFs, miRNAs and genes based on the experimentally verified or predicted data. Secondly, the FFLs and FBLs are identified by the relations obtained in the first step. Finally, the TF-miRNA co-regulatory network is constructed by merging the FFLs and FBLs together.

miRNAs and TFs. TFs of E2F family are activated at the G1/S phase boundary and play critical roles in controlling cell cycle progression by regulating the timely expression of genes required for DNA synthesis [53]. E2F1, one member of E2F family, can directly activate the transcription of miR-17-92 cluster, which in turn represses its translation [54] (Figure 3). Similar interactions have been proposed for E2F1 and miR-17-92 paralog clusters miR-106b-25 and miR-106a-363 [55,57]. Thus, E2F1 stimulates its own transcription in a positive autoregulatory loop and prevents its excessive accumulation in negative FBLs by activating miR-17-92 and its paralog clusters (Figure 3). E2F1 activity is repressed by pocket proteins (e.g. pRb and p130). This repression can be released by miRNAs in the above clusters through silencing key members of the E2F pathway such as CDK inhibitors (e.g. p21 and p57) and pocket proteins. However, this release is repressed by E2F1 itself through activating these genes (Figure 3) [55]. In summary, E2F1 balances its protein level and activity through forming single-negative FBLs and incoherent FFLs with miRNAs (Fig. 3). These incoherent loops regulate the protein level of E2F1 to achieve optimal activity and ensure the progression of cell cycle. Furthermore, these miRNA clusters also impart a buffering effect, where they protect E2F1 activation against fluctuating signals.

However, the balancing and buffering effect between E2F1 and miRNAs in this network can be broken by oncogene c-Myc or tumor suppressor p53 (Figure 3). Several studies reported that c-Myc could activate the expression of members from the above miRNA clusters, and then lead to the deregulated and hyperactive E2Fs in cancer cells [18,56,58]. For example, c-Myc activates miR-17 and miR-20a to avoid a G1 checkpoint arising from the untimely accumulation of E2F1 [58]. Tagawa *et al.* [59] reported that the enforced expression of the miR-17-92 cluster cooperates with c-Myc in mice to promote formation of B-cell lymphomas. In addition, c-Myc promotes the progress of the cell cycle through forming a positive FBL with E2F1 directly [60]. P53 indirectly inhibits E2F1 through different ways, including activating the expression of CDK inhibitors and activating the miR-34 family members, which target crucial cell-cycle effectors such as Cdk4, Cdk6, cyclin E2 and E2Fs [17,61]. Ran Brosh *et al.* [55] elucidated that p53 repress the expression of miRNAs in these three

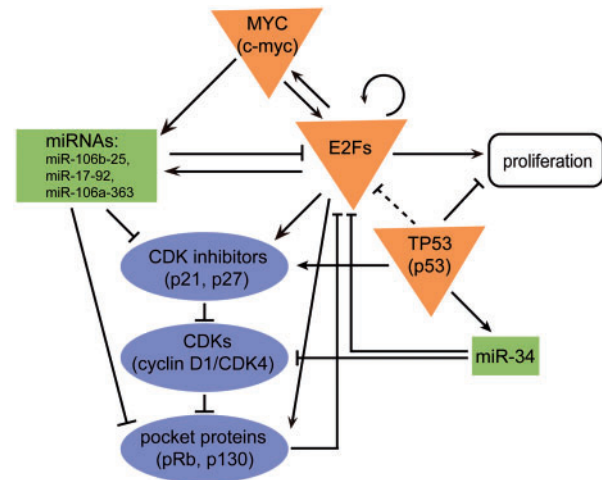


Figure 3: A schematic model for TF-miRNA co-regulatory network in cell proliferation. The E2F family and three miRNA clusters form several composite FFLs with CDK inhibitors and pocket proteins. They corporately control the progression of the cell cycle. The oncogene c-Myc can promote cell cycle progress through directly activating the E2F family and miRNA clusters, while the tumor repressor p53 represses E2Fs activity in an indirect way. The meanings of sharp arrows and T-shaped solid arrows are same as Figure 1. T-shaped dotted arrow indicates the indirect repression of P53 to E2Fs. This figure is drawn based on two previous articles [55,56].

clusters in an E2F1-mediated manner. P53 inactivation or overexpression of representative miRNAs of these three clusters promotes cell proliferation and delays senescence.

TF-miRNA CO-REGULATION IN CELL DIFFERENTIATION AND DEVELOPMENT

The combined TF-miRNA regulatory motifs, especially FBLs, have emerged as popular regulatory mechanisms in cell differentiation and development. In this section, we will introduce the important functions of FBLs and FFLs using the following identified examples.

FBL in epithelial-mesenchymal transition

The EMT occurs during embryologic development and adult tissue remodeling, and is viewed as an essential early step in the metastasis of epithelial-derived tumors [62,63]. MiR-200 family and TFs ZEB1 and SIP1 (also known as ZEB2) induce this transition through establishing a double-negative

FBL (Figure 4a). In epithelial cells, a stable state is maintained by the high levels of miR-200 family, which inhibits the expression of ZEB1/SIP1. Thus, miR-200 family increases the expression of ZEBs-repressed epithelial genes such as E-cadherin, which plays a crucial role in the cell–cell adhesion and the maintenance of tissue architecture. In turn, a mesenchymal state is instigated through high expression of ZEB1/SIP1 in response to the transforming growth factor beta (TGF β) induction [15,64], which will repress miR-200 family. Once the levels of miR-200 fall below a threshold, the mesenchymal phenotype will be maintained.

FBL in skeletal myogenesis

It is reported that some miRNAs and TFs are involved in muscle development and muscle-related diseases. Among them, TF Yin Yang 1 (YY1) and two miRNAs (miR-1 and miR-29) play critical roles in skeletal myogenesis through forming double-negative FBLs (Figure 4b) [16,65]. In myoblasts, the constitutive activity of NF- κ B activates YY1 expression, which subsequently suppresses two inducers of myogenesis miR-1 and miR-29. The downregulation of miR-1 releases Pax7, which is required for satellite cell proliferation and prevents myogenesis (Figure 4b) [66]. Thus, the high expression of YY1 maintains myoblasts in an undifferentiated state. At the onset of myogenesis, YY1 is downregulated by decreased NF- κ B signaling, which leads to the upregulation of miR-1 and miR-29. In turn, miR-1 and miR-29 further decreases the expression of YY1. Pax7 also is downregulated by miR-1 (Figure 4b). Thus, the differentiation from myoblasts into myotubes is ensured by the high expression of miR-1 and miR-29 [16].

FBL in neuronal cell fate decision

Taste receptor neurons of *Caenorhabditis elegans* have two alternative fates ‘ASE left’ (ASEL) and ‘ASE right’ (ASER), which share many bilaterally symmetric features. These two terminally differentiated states require the activity of a set of regulatory factors that interact with one another in a bistable, double-negative FBL [67,68]. In ASEL neurons, the ASEL-specific inducer genes (TF die-1 and miRNA lsy-6) activate other ASEL-specific effectors (lim-6, flp-4, flp-20 etc.) and repress ASER-inducers (TF cog-1 and mir-273) (Figure 4c), which determines the stable state of ASEL. Similarly, in ASER neurons, ASER-inducer genes (TF cog-1 and mir-273) are

expressed and maintain the stable ASER state through activating ASER effectors (gcy-5, gcy-22, and hen-1) and repressing the ASEL-inducer genes (TF die-1 and miRNA lsy-6) (Figure 4c). As a result, the inducer genes of ASEL and ASER jointly form a double-negative FBL, which affects the neuronal cell fates decision with ASEL and ASER effectors [67].

FBL in midbrain dopaminergic neuron maturation

Midbrain dopaminergic neurons (DNs) are the main source of dopamine in the mammalian central nervous system and these cells are lost in Parkinson’s disease [69]. Kim *et al.* [70] identified that miR-133b is specifically expressed in midbrain DN and regulates the maturation and function of midbrain DN within a single-negative FBL. In this FBL, miR-133b is induced by Pitx3 and in turn downregulates Pitx3 activity (Figure 4d). Such kind of FBL has been shown to speed response time and provide the stability in the context of dynamic changes [71]. Midbrain DN function is dynamic, thereby this FBL limits the fluctuant ranges over which the concentrations of Pitx3 and miR-133b.

FBL in cardiogenesis

Cardiogenesis is regulated by serum response factor (SRF) and miR-133a-2 in a single-negative FBL [72]. The TF SRF is able to enhance the expression of miR-133a-2, which in turn represses the level of SRF (Figure 4e). Niu *et al.* [72] have found that SRF activity is tightly regulated through this miR-133a-2-dependent negative FBL and the delicate balance of miR-133a-2-directed silencing of SRF activity is profound in human heart disease.

FBL in photoreceptor differentiation

The double-negative FBL is prevalent in cell differentiation and development. However, it brings an intrinsic risk that stochastic fluctuations make cells rapidly flip between alternative cell decisions [8]. Robustness is imbued by the coherent FFLs, which has been characterized in photoreceptors differentiation of *Drosophila melanogaster* [11]. The miR-7 and TF Yan control this differentiation process in a FFL way. In progenitor cells, Yan is activated by Notch signaling and then represses miR-7 directly. Yan also repress miR-7 transcription indirectly through Ttk69, a transcription repressor, which is targeted by Phyl ubiquitin ligase (Figure 4f). Thus, the high expression of Yan stabilizes the progenitor cells state. EGF

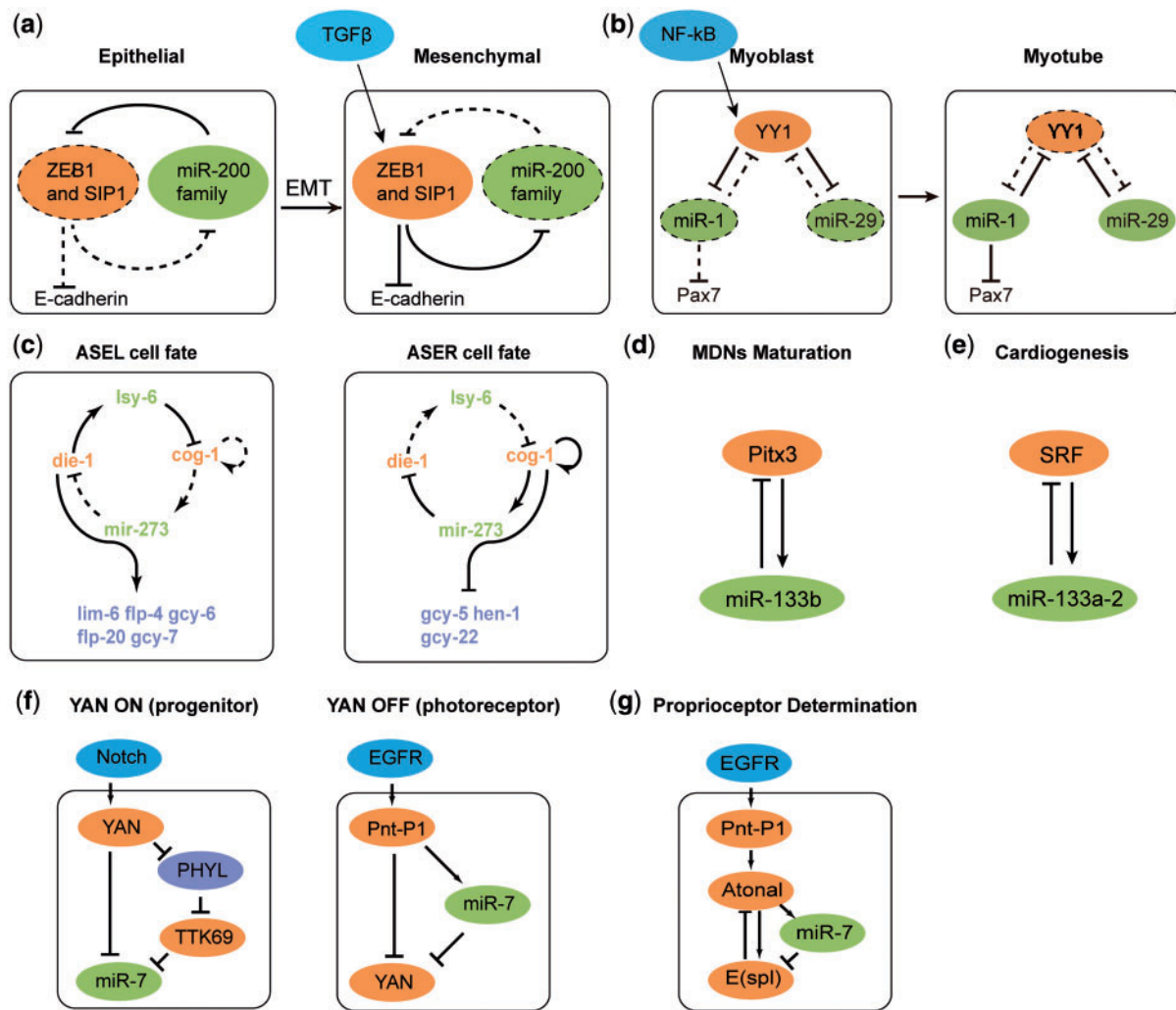


Figure 4: FFLs and FBLs in cell differentiation. Orange ovals are TFs; green ovals are miRNAs; light blue ovals are upstream signals. Dotted line means the activation or repression is inactive; dotted oval means the gene or miRNA is repressed or in a low expression. (a) The FBL between TFs ZEB1/SIP1 and miR-200 family in EMT. In epithelial cells, ZEB1 and SIP1 are repressed by miR-200 family. EMT is induced when ZEB1 and SIP1 are activated by the TGF β signal and miR-200 family is repressed. (b) The FBLs in skeletal myogenesis. The high expression of TF YY1 activated by NF- κ B signal maintains the undifferentiated states of myoblast cells. At the onset of myogenesis, the down-regulation of the NF- κ B-YY1 pathway leads to an upregulation of miR-1 and miR-29, which ensures myoblast cells properly differentiate into myotubes. (c) The FBL in neuronal cell differentiation. The high expression of TF die-1 and miRNA lsy-6 or TF cog-1 and miR-273 determines the taste receptor neurons of *C. elegans* to differentiate into 'ASE left' or 'ASE right'. (d) The FBL in midbrain dopaminergic neurons (MDNs). MiR-133b is induced by TF Pitx3 and in turn down-regulates Pitx3 activity. (e) The FBL in cardiogenesis. The activity of TF SRF is tightly regulated through this miR-133a-2 dependent single negative FBL. (f) Two FFLs in the differentiation of photoreceptor. The expression level of TF YAN determines this differentiation process. (g) The FFL in proprioceptor determination. The sustained activation of EGFR signaling to TF Ato results in sustained repression of TF E(spl) by miR-7 and the stable expression of Ato, and thus induces the proprioceptor determination. A colour version of this figure is available at BIB online: <http://bib.oxfordjournals.org>.

signaling induces progenitor cells to differentiate into photoreceptors by activating another coherent FFL: Pnt-P1 directly represses Yan and activates miR-7, which in turn represses Yan (Figure 4f). This FFL upregulates miR-7 and promotes Yan degradation, relieving miR-7 from repression. Coherent FFLs of

this type, in which X activates Y, and both of them repress Z, generates a delay or persistence that rejects fluctuations in X [73]. This buffering stabilizes cellular responses and ensures that the cell switches from one state (Yan ON) to the other state (Yan OFF) when there is a persistent decrease in Yan [11].

FFL in proprioceptor determination

Precursor cells of proprioceptor transiently express the *Atonal* (*Ato*) gene in proneural cluster (PNC), which enables a subset of PNC cells to adopt a sensory organ precursor (SOP) fate, and SOPs then proceed to form the sensory organs. The process of SOP determination is controlled by two inducers miR-7 and *Ato* and one repressor *E(spl)* in an incoherent FFL (Figure 4g). In this loop, *Ato* directly activates the transcription of *E(spl)*, and also indirectly represses *E(spl)* expression through activating miR-7. In addition, *E(spl)* feeds back to *Ato* to create a single-negative FBL and is interconnected with the FFL. In this network, the sustained activation of *Ato* by EGFR signaling results in a sustained repression of *E(spl)* by miR-7 and a stable expression of *Ato*, which induces the SOP determination [11].

TF-miRNA CO-REGULATION IN DISEASES

The abnormal expression of TF and miRNA is usually associated with diseases. In cancers, miRNAs and TFs can function as oncogenes or tumor suppressors in a cooperative way, thus triggering global alteration of gene expression. In this part, we will introduce the functions of TF-miRNA FFLs and FBLs in cancers and other diseases.

Cancers

MiRNA and TF are important regulators in all kinds of cancers. However, their FFLs and FBLs were only analyzed in a few of well-studied cancers. Acute myeloid leukemia (AML) is characterized by the rapid growth of hematopoietic precursors blocked at different differentiation stages, such as erythroid, or granulocytic [74]. Two studies reported that miR-223 and three TFs (*C/EBP α* , *NFI-A* and *E2F1*) play critical roles in granulocyte differentiation and the occurrence of AML (Figure 5a) [75,76]. Fazi *et al.* [76] elucidated granulopoiesis is controlled by miR-223 and two TFs *C/EBP α* and *NFI-A* through forming a regulatory circuit. In promyelocytic cells, the *NFI-A* maintains low miR-223 expression through binding to the miR-223 promoter. During granulopoiesis, *C/EBP α* is highly expressed, which replaces *NFI-A* from the miR-223 promoter and upregulates miR-223 expression. In turn, miR-223 represses *NFI-A*, thus subtracting it from the competition with *C/EBP α* and maintaining

sustained high level of miR-223 expression. On the other hand, miR-223 represses the translation of *E2F1* resulting in inhibition of cell-cycle progression and myeloid differentiation [75]. However, *C/EBP α* is deregulated in AML and the activation of miR-223 is inhibited, which results in accumulation of *E2F1*. Moreover, overexpressed *E2F1* inhibits miR-223 transcription, thus promoting myeloid cell proliferation and blocking differentiation [75]. Thus, miR-223 as a molecular switch is regulated by *C/EBP α* and *E2F1* in granulopoiesis and in AML, respectively. It could be a therapeutic target for AML subtypes in which *E2F1* inhibition is dysregulated [75].

T-cell acute lymphoblastic leukemia (T-ALL) is a highly aggressive hematologic malignancy. To study the regulatory mechanism, we constructed a TF-miRNA co-regulatory network specific for T-ALL through collecting T-ALL-related genes, miRNAs and TFs [51]. MiR-19 is an oncogene inhibiting the expression of tumor suppressor genes, such as *PTEN* and *BCL2L11*, and is overexpressed in T-ALL patients. MiR-19 and *CYLD*, as hubs in this network, form an incoherent FFL with *NF- κ B*, in which *NF- κ B* activates the transcription of miR-19 and *CYLD*, and miR-19 represses the translation of *CYLD*. In addition, *CYLD* can reduce the nuclear translocation of *NF- κ B* (Figure 5b). This FFL suggests that miR-19 and *CYLD* play critical regulatory roles in the *NF- κ B* pathway and provides a new mechanism for the sustained activation of *NF- κ B* in T-ALL. *NF- κ B* pathway has been proposed as a potential target for therapies of T-ALL [77].

Breast cancer is a common disease among women. MiR-34a acts as a tumor suppressor by blocking cell cycle arrest to G1-phase and inducing cell senescence and apoptosis in breast, colon, lung and other cell lines [61,78]. It is highly expressed in normal tissues and is reduced in several cancer types [79,80]. However, Peurala *et al.* [81] found that miR-34a has a high or medium expression in most of the breast cancer patients, which is associated with a lower risk of recurrence or death from breast cancer. The transcription of miR-34a is modulated by two TFs *P53* and *MAZ*, and both of them can form FFLs with miR-34a and target genes (Figure 5c). Many of their targets are cancer-related genes, which are involved in several cellular pathways such as 'cell death' and 'cell cycle'. The target genes co-regulated by miR-34a, *P53* and *MAZ* might be used in predicting clinical outcome [81].

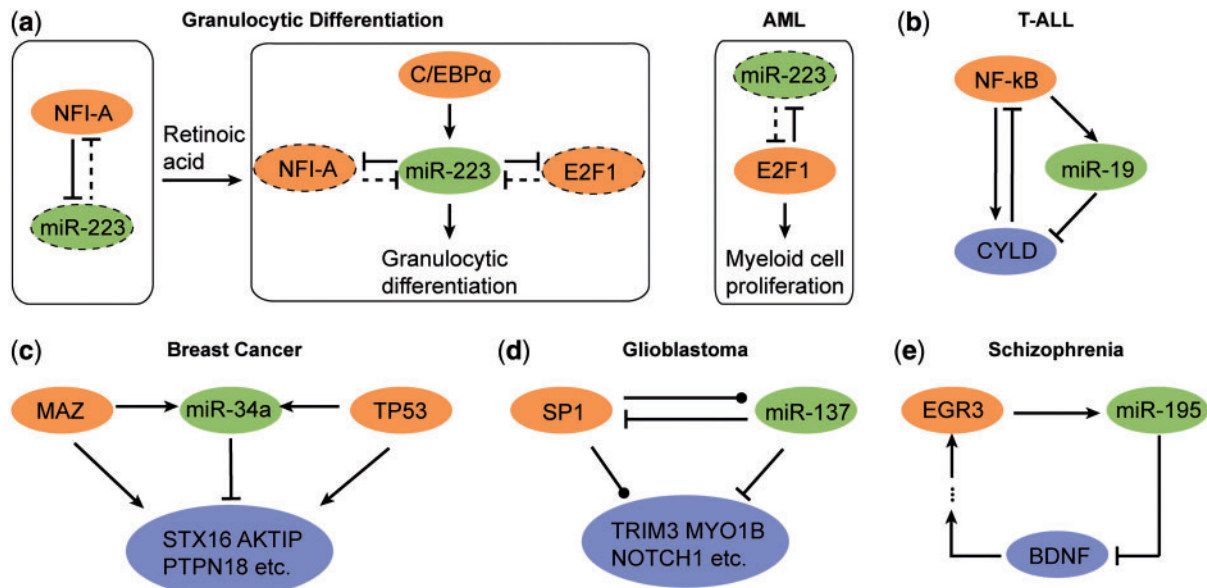


Figure 5: FFLs and FBLs in diseases. (a) The FBL in granulocytic differentiation and myeloid cell proliferation. In the undifferentiated cells, TF NFI-A maintains the miR-223 at low level. The TF C/EBP α is activated by retinoic acid and upregulates miR-223 expression, which in turn represses TFs NFI-A and E2F1, resulting in inhibition of cell cycle and advance of granulocytic differentiation (left). C/EBP α is deregulated in AML and overexpressed E2F1 inhibits miR-223 transcription, thus promoting myeloid cell proliferation and blocking granulocytic differentiation (right). (b) A FFL in T-ALL. (c) The predicted FFLs in breast cancer. (d) A predicted FFL in glioblastoma. (e) A FFL in schizophrenia. TF EGR3 activates the transcription of miR-195, and in turn miR-195 indirectly reduces the expression of EGR3 by repressing gene BDNF.

Ovarian cancer is a malignant gynecologic neoplasm. Researchers have inferred TF-miRNA co-regulatory networks associated with its survival and recurrence through the simultaneous analysis of co-expression profiles and clinical characteristics of patients. In these networks, the aberrant expression of three miRNAs (miR-16, miR-22-5p and ebv-miR-BHRF1-2-5p), four TFs (FOS, EGR2, EGR1 and TGFB1) and many of their targets are associated with the survival and recurrence. Some of these molecules may be important biomarkers for the diagnosis of ovarian cancer [82].

Glioblastoma multiforme (GBM) is the most common and highest-grade brain tumor. Sun *et al.* have constructed a comprehensive GBM-specific TF-miRNA co-regulatory network through compiling GBM-related molecules and identified many 3-node and 4-node FFLs with statistical significance. In addition, they generated a subnetwork for the Notch signaling pathway, in which four TFs (EP300, SP1, TEAD1 and TBX5) and four miRNAs (miR-137, miR-34a, miR-9 and miR-92b) act as hubs. Figure 5d gives an example of the high-order subnetworks that form the GBM-specific TF-miRNA co-regulatory network [83].

Other diseases

Schizophrenia is a complex and severe mental disorder. Its pathophysiology and molecular mechanisms have remained poorly understood [84]. In our previous study, we analyzed the schizophrenia-specific TF-miRNA regulatory network, in which TF EGR3 and miR-195 are core regulators. In our result, we found that EGR3 is induced by BDNF via a PKC/MAPK-dependent pathway, and then EGR3 activates the transcription of miR-195, which in turn inhibits BDNF expression. Thus, EGR3, has-miR-195 and BDNF form a critical feed-back regulatory loop (Figure 5e) [19]. This FBL implies that EGR3 negatively regulates its own transcription. The expression of EGR3 is decreased in schizophrenia patients [85] and it is a downstream gene of many other signaling pathways. Thus, EGR3 maybe play important roles in schizophrenia.

Interstitial lung disease (ILD) is a term that describes a broad category of restrictive lung disorders, which is associated with the dysregulation of many regulators and signaling pathways including TGF β [86]. Cho *et al.* [87] conducted a system analysis of the molecular networks of ILD by identifying FFLs from the differentially expressed miRNAs, TFs

and genes. ZEB1 and miR-23a, as the top hubs of this network, play key roles in promoting disease progression. ZEB1, an inducer of the EMT, activates the transcription of miR-23a, which in turn represses the translation of NEDD4L. NEDD4L, an ubiquitin ligase, downregulates TGF β pathway activity by triggering Smad2/3 and Tgfbr1 ubiquitination. Thus, ZEB1-mediated EMT stabilizes or enhances TGF β signaling and promotes disease progression through activating the miR-23a clusters' regulation of Nedd4L protein levels [87].

CONCLUSIONS AND FUTURE CHALLENGES

The TF-miRNA co-regulatory network undoubtedly provides a comprehensive view of gene expression regulation at a systems level. It will be more powerful once reliable and complete data of miRNA and TF targets is incorporated. For the identification of miRNA targets, the high-throughput techniques CLIP-seq (cross-linking immunoprecipitation high-throughput sequencing) and CLASH (cross-linking, ligation and sequencing of hybrids) speed up this process. The CLASH technique developed by Helwak *et al.* [88] ligates and sequences miRNA-target RNA duplexes, thus can detect miRNA targets in a more accurate and high-throughput way. In addition, this work found that about half of the miRNA binding sites are not in mRNA 3' untranslated regions (UTRs) but in the 5' UTRs and coding sequence (CDS) regions based on the CLASH technology [88]. Thus, the miRNA target prediction and validation are still an urgent issue. Studies about TF target identification are also insufficient, although ChIP-seq and other methods accelerate the identification of TF targets. Much work has been done to reduce the false positives in ChIP-seq, but an integration of the ChIP-seq result with corresponding perturbed expression profiles of TF will make it more reliable.

To date, computational TF-miRNA regulatory networks are available for some genomes and many diseases [4,19,51,82]. However, experimentally verified regulations are only a small part of all the predicted network motifs. It is critical to speed up the confirmation of TF-miRNA co-regulatory networks to study their functions. Besides that, it is difficult to detect the powers of FFLs or FBLs in regulating gene expression or their impacts in diseases. As a trial to address this problem, the web sever dChip-GemINI

calculates the network motif score and the false discovery rate for each candidate TF-miRNA FFL and ranks them by their explanatory power to account for the differential expression of genes and miRNAs [21].

It is context-dependent for TF and miRNA regulating gene expression. The ChIP-seq results have shown that each TF can target various genes in different cell lines [89]. Neph *et al.* [90] also found that human TF networks are highly cell selective through analyzing the dynamics of 475 TFs across 41 diverse cell and tissue types. To obtain an accurate analysis of gene expression, it is essential to combine all the data from expression profiles of miRNAs and TFs, the high-throughput experiments of TFs, miRNAs target detection and the TF-miRNA co-regulatory network.

It will be crucial to integrate TF-miRNA regulatory networks with other functional networks, such as signaling pathways, metabolic pathways, protein-protein interaction networks and co-expressed signatures [20,91]. This integration will aid in explaining how these networks regulate the biological processes and diseases at the systems level.

Key Points

- TFs and miRNAs can jointly regulate gene expression in the forms of FFLs and FBLs, which influence many aspects of normal cells and diseases.
- FFLs and FBLs can be classified into different types based on the master regulator or the regulation effects of two paths on target. Different types of loops have different mechanisms in gene regulation.
- The identification of TF and miRNA targets is a key step for detecting FFLs and FBLs. It is better to combine the experimentally verified targets with predicted targets by different methods.
- FFLs and FBLs are popular regulatory models and critical for biological processes and diseases. FFL has a specific function in noise buffering effect. It can minimize the cell response to stochastic signaling noise and maintain steady-state levels of targets. FBL can act as a toggle switch between two different fates in cell differentiation.

Acknowledgements

We would like to thank Hu Chen, Vusumuzi Leroy Sibanda and Hui Liu for valuable suggestions and discussion on this work.

FUNDING

National Natural Science Foundation of China (NSFC) (31171271, 31270885) and Program for New Century Excellent Talents in University (NCET), Ministry of Education of China.

References

1. Lee TI, Young RA. Transcription of eukaryotic protein-coding genes. *Annu Rev Genet* 2000;**34**:77–137.
2. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004;**116**:281–97.
3. Shalgi R, Lieber D, Oren M, et al. Global and local architecture of the mammalian microRNA–transcription factor regulatory network. *PLoS Comput Biol* 2007;**3**:e131.
4. Tsang J, Zhu J, van Oudenaarden A. MicroRNA-mediated feedback and feedforward loops are recurrent network motifs in mammals. *Mol Cell* 2007;**26**:753–67.
5. Hirashima T, Iwasa Y, Morishita Y. Distance between AER and ZPA is defined by feed-forward loop and is stabilized by their feedback loop in vertebrate limb bud. *Bull Math Biol* 2008;**70**:438–59.
6. Kalir S, Mangan S, Alon U. A coherent feed-forward loop with a SUM input function prolongs flagella expression in *Escherichia coli*. *Mol Syst Biol* 2005;**1**:2005.0006.
7. Martinez NJ, Ow MC, Barrasa MI, et al. A *C. elegans* genome-scale microRNA network contains composite feedback motifs with high flux capacity. *Genes Dev* 2008;**22**:2535–49.
8. Inui M, Martello G, Piccolo S. MicroRNA control of signal transduction. *Nat Rev Mol Cell Biol* 2010;**11**:252–63.
9. Shalgi R, Brosh R, Oren M, et al. Coupling transcriptional and post-transcriptional miRNA regulation in the control of cell fate. *Aging (Albany NY)* 2009;**1**:762–70.
10. Bartel DP, Chen CZ. Micromanagers of gene expression: the potentially widespread influence of metazoan microRNAs. *Nat Rev Genet* 2004;**5**:396–400.
11. Li X, Cassidy JJ, Reinke CA, et al. A microRNA imparts robustness against environmental fluctuation during development. *Cell* 2009;**137**:273–82.
12. Osella M, Bosia C, Cora D, et al. The role of incoherent microRNA-mediated feedforward loops in noise buffering. *PLoS Comput Biol* 2011;**7**:e1001101.
13. Shen-Orr SS, Milo R, Mangan S, et al. Network motifs in the transcriptional regulation network of *Escherichia coli*. *Nat Genet* 2002;**31**:64–8.
14. Alon U. Network motifs: theory and experimental approaches. *Nat Rev Genet* 2007;**8**:450–61.
15. Bracken CP, Gregory PA, Kolesnikoff N, et al. A double-negative feedback loop between ZEB1-SIP1 and the microRNA-200 family regulates epithelial-mesenchymal transition. *Cancer Res* 2008;**68**:7846–54.
16. Lu L, Zhou L, Chen EZ, et al. A Novel YY1-miR-1 regulatory circuit in skeletal myogenesis revealed by genome-wide prediction of YY1-miRNA network. *PLoS One* 2012;**7**:e27596.
17. Bommer GT, Gerin I, Feng Y, et al. p53-mediated activation of miRNA34 candidate tumor-suppressor genes. *Curr Biol* 2007;**17**:1298–307.
18. O'Donnell KA, Wentzel EA, Zeller KI, et al. c-Myc-regulated microRNAs modulate E2F1 expression. *Nature* 2005;**435**:839–43.
19. Guo AY, Sun J, Jia P, et al. A novel microRNA and transcription factor mediated regulatory network in schizophrenia. *BMC Syst Biol* 2010;**4**:10.
20. Sun J, Gong X, Purow B, et al. Uncovering MicroRNA and transcription factor mediated regulatory networks in glioblastoma. *PLoS Comput Biol* 2012;**8**:e1002488.
21. Yan Z, Shah PK, Amin SB, et al. Integrative analysis of gene and miRNA expression profiles with transcription factor-miRNA feed-forward loops identifies regulators in human cancers. *Nucleic Acids Res* 2012;**40**:e135.
22. Bisognin A, Sales G, Coppe A, et al. MAGIA²: from miRNA and genes expression data integrative analysis to microRNA–transcription factor mixed regulatory circuits (2012 update). *Nucleic Acids Res* 2012;**40**:W13–21.
23. Huang GT, Athanassiou C, Benos PV. mirConnX: condition-specific mRNA-microRNA network integrator. *Nucleic Acids Res* 2011;**39**:W416–23.
24. Le Beche A, Portales-Casamar E, Vetter G, et al. MIR@NT@N: a framework integrating transcription factors, microRNAs and their targets to identify sub-network motifs in a meta-regulation network model. *BMC Bioinformatics* 2011;**12**:67.
25. Friard O, Re A, Taverna D, et al. CircuitsDB: a database of mixed microRNA/transcription factor feed-forward regulatory circuits in human and mouse. *BMC Bioinformatics* 2010;**11**:435.
26. El Baroudi M, Cora D, Bosia C, et al. A curated database of miRNA mediated feed-forward loops involving MYC as master regulator. *PLoS One* 2011;**6**:e14742.
27. Wang J, Lu M, Qiu C, et al. TransmiR: a transcription factor-microRNA regulation database. *Nucleic Acids Res* 2010;**38**:D119–22.
28. Vaquerizas JM, Kummerfeld SK, Teichmann SA, et al. A census of human transcription factors: function, expression and evolution. *Nat Rev Genet* 2009;**10**:252–63.
29. Zhang HM, Chen H, Liu W, et al. AnimalTFDB: a comprehensive animal transcription factor database. *Nucleic Acids Res* 2012;**40**:D144–9.
30. Qin J, Li MJ, Wang P, et al. ChIP-Array: combinatory analysis of ChIP-seq/chip and microarray gene expression data to discover direct/indirect targets of a transcription factor. *Nucleic Acids Res* 2011;**39**:W430–6.
31. Matys V, Kel-Margoulis OV, Fricke E, et al. TRANSFAC and its module TRANSCOMP: transcriptional gene regulation in eukaryotes. *Nucleic Acids Res* 2006;**34**:D108–10.
32. Lachmann A, Xu H, Krishnan J, et al. ChEA: transcription factor regulation inferred from integrating genome-wide ChIP-X experiments. *Bioinformatics* 2010;**26**:2438–44.
33. Wang J, Zhuang J, Iyer S, et al. Factorbook.org: a Wiki-based database for transcription factor-binding data generated by the ENCODE consortium. *Nucleic Acids Res* 2013;**41**:D171–6.
34. Bryne JC, Valen E, Tang MH, et al. JASPAR, the open access database of transcription factor-binding profiles: new content and tools in the 2008 update. *Nucleic Acids Res* 2008;**36**:D102–6.
35. Lee Y, Kim M, Han J, et al. MicroRNA genes are transcribed by RNA polymerase II. *EMBOJ* 2004;**23**:4051–60.
36. Saini HK, Griffiths-Jones S, Enright AJ. Genomic analysis of human microRNA transcripts. *Proc Natl Acad Sci USA* 2007;**104**:17719–24.
37. Isik M, Korswagen HC, Berezikov E. Expression patterns of intronic microRNAs in *Caenorhabditis elegans*. *Silence* 2010;**1**:5.

38. Baskerville S, Bartel DP. Microarray profiling of microRNAs reveals frequent coexpression with neighboring miRNAs and host genes. *RNA* 2005;**11**:241–7.
39. He C, Li Z, Chen P, *et al.* Young intragenic miRNAs are less coexpressed with host genes than old ones: implications of miRNA–host gene coevolution. *Nucleic Acids Res* 2012;**40**:4002–12.
40. Garcia DM, Baek D, Shin C, *et al.* Weak seed-pairing stability and high target-site abundance decrease the proficiency of *lcy-6* and other microRNAs. *Nat Struct Mol Biol* 2011;**18**:1139–46.
41. Betel D, Koppal A, Agius P, *et al.* Comprehensive modeling of microRNA targets predicts functional non-conserved and non-canonical sites. *Genome Biol* 2010;**11**:R90.
42. Kertesz M, Iovino N, Unnerstall U, *et al.* The role of site accessibility in microRNA target recognition. *Nat Genet* 2007;**39**:1278–84.
43. Chen K, Rajewsky N. Natural selection on human microRNA binding sites inferred from SNP data. *Nat Genet* 2006;**38**:1452–6.
44. Kruger J, Rehmsmeier M. RNAhybrid: microRNA target prediction easy, fast and flexible. *Nucleic Acids Res* 2006;**34**:W451–4.
45. Hsu SD, Chu CH, Tsou AP, *et al.* miRNAMap 2.0: genomic maps of microRNAs in metazoan genomes. *Nucleic Acids Res* 2008;**36**:D165–9.
46. Le Brigand K, Robbe-Sermesant K, Mari B, *et al.* MiRonTop: mining microRNAs targets across large scale gene expression studies. *Bioinformatics* 2010;**26**:3131–2.
47. Vergoulis T, Vlachos IS, Alexiou P, *et al.* TarBase 6.0: capturing the exponential growth of miRNA targets with experimental support. *Nucleic Acids Res* 2012;**40**:D222–9.
48. Hsu SD, Lin FM, Wu WY, *et al.* miRTarBase: a database curates experimentally validated microRNA–target interactions. *Nucleic Acids Res* 2011;**39**:D163–9.
49. Xiao F, Zuo Z, Cai G, *et al.* miRecords: an integrated resource for microRNA–target interactions. *Nucleic Acids Res* 2009;**37**:D105–10.
50. Jiang Q, Wang Y, Hao Y, *et al.* miR2Disease: a manually curated database for microRNA deregulation in human disease. *Nucleic Acids Res* 2009;**37**:D98–104.
51. Ye H, Liu X, Lv M, *et al.* MicroRNA and transcription factor co-regulatory network analysis reveals miR-19 inhibits CYLD in T-cell acute lymphoblastic leukemia. *Nucleic Acids Res* 2012;**40**:5201–14.
52. Shannon P, Markiel A, Ozier O, *et al.* Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* 2003;**13**:2498–504.
53. DeGregori J. The genetics of the E2F family of transcription factors: shared functions and unique roles. *Biochim Biophys Acta* 2002;**1602**:131–50.
54. Woods K, Thomson JM, Hammond SM. Direct regulation of an oncogenic micro-RNA cluster by E2F transcription factors. *J Biol Chem* 2007;**282**:2130–4.
55. Brosh R, Shalgi R, Liran A, *et al.* p53-Repressed miRNAs are involved with E2F in a feed-forward loop promoting proliferation. *Mol Syst Biol* 2008;**4**:229.
56. Knoll S, Emmrich S, Putzer BM. The E2F1–miRNA cancer progression network. *Adv Exp Med Biol* 2013;**774**:135–47.
57. Petrocca F, Vecchione A, Croce CM. Emerging role of miR-106b-25/miR-17-92 clusters in the control of transforming growth factor beta signaling. *Cancer Res* 2008;**68**:8191–4.
58. Pickering MT, Stadler BM, Kowalik TF. miR-17 and miR-20a temper an E2F1-induced G1 checkpoint to regulate cell cycle progression. *Oncogene* 2009;**28**:140–5.
59. Tagawa H, Karube K, Tsuzuki S, *et al.* Synergistic action of the microRNA-17 polycistron and Myc in aggressive cancer development. *Cancer Sci* 2007;**98**:1482–90.
60. Matsumura I, Tanaka H, Kanakura Y. E2F1 and c-Myc in cell growth and death. *Cell Cycle* 2003;**2**:333–8.
61. Tazawa H, Tsuchiya N, Izumiya M, *et al.* Tumor-suppressive miR-34a induces senescence-like growth arrest through modulation of the E2F pathway in human colon cancer cells. *Proc Natl Acad Sci USA* 2007;**104**:15472–7.
62. Chaffer CL, Weinberg RA. A perspective on cancer cell metastasis. *Science* 2011;**331**:1559–64.
63. Kong D, Li Y, Wang Z, *et al.* Cancer stem cells and epithelial-to-mesenchymal transition (EMT)–phenotypic cells: are they cousins or twins? *Cancers (Basel)* 2011;**3**:716–29.
64. Gregory PA, Bert AG, Paterson EL, *et al.* The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. *Nat Cell Biol* 2008;**10**:593–601.
65. Wang H, Garzon R, Sun H, *et al.* NF-kappaB-YY1-miR-29 regulatory circuitry in skeletal myogenesis and rhabdomyosarcoma. *Cancer Cell* 2008;**14**:369–81.
66. Olguin HC, Olwin BB. Pax-7 up-regulation inhibits myogenesis and cell cycle progression in satellite cells: a potential mechanism for self-renewal. *Dev Biol* 2004;**275**:375–88.
67. Johnston RJ, Jr, Chang S, Etchberger JF, *et al.* MicroRNAs acting in a double-negative feedback loop to control a neuronal cell fate decision. *Proc Natl Acad Sci USA* 2005;**102**:12449–54.
68. Johnston RJ, Jr, Hobert O. A novel *C. elegans* zinc finger transcription factor, *lcy-2*, required for the cell type-specific expression of the *lcy-6* microRNA. *Development* 2005;**132**:5451–60.
69. Ang SL. Transcriptional control of midbrain dopaminergic neuron development. *Development* 2006;**133**:3499–506.
70. Kim J, Inoue K, Ishii J, *et al.* A MicroRNA feedback circuit in midbrain dopamine neurons. *Science* 2007;**317**:1220–4.
71. Becskei A, Serrano L. Engineering stability in gene networks by autoregulation. *Nature* 2000;**405**:590–3.
72. Niu Z, Li A, Zhang SX, *et al.* Serum response factor micro-managing cardiogenesis. *Curr Opin Cell Biol* 2007;**19**:618–27.
73. Mangan S, Alon U. Structure and function of the feed-forward loop network motif. *Proc Natl Acad Sci USA* 2003;**100**:11980–5.
74. Tenen DG. Disruption of differentiation in human cancer: AML shows the way. *Nat Rev Cancer* 2003;**3**:89–101.
75. Pulikkan JA, Dengler V, Peramangalam PS, *et al.* Cell-cycle regulator E2F1 and microRNA-223 comprise an autoregulatory negative feedback loop in acute myeloid leukemia. *Blood* 2010;**115**:1768–78.
76. Fazi F, Rosa A, Fatica A, *et al.* A minicircuitry comprised of microRNA-223 and transcription factors NFI-A and C/EBPalpha regulates human granulopoiesis. *Cell* 2005;**123**:819–31.

77. Vilimas T, Mascarenhas J, Palomero T, *et al.* Targeting the NF-kappaB signaling pathway in Notch1-induced T-cell leukemia. *Nat Med* 2007;**13**:70–77.
78. Tarasov V, Jung P, Verdoodt B, *et al.* Differential regulation of microRNAs by p53 revealed by massively parallel sequencing: miR-34a is a p53 target that induces apoptosis and G1-arrest. *Cell Cycle* 2007;**6**:1586–93.
79. Corney DC, Hwang CI, Matoso A, *et al.* Frequent down-regulation of miR-34 family in human ovarian cancers. *Clin Cancer Res* 2010;**16**:1119–28.
80. Dijkstra MK, van Lom K, Tielemans D, *et al.* 17p13/TP53 deletion in B-CLL patients is associated with microRNA-34a downregulation. *Leukemia* 2009;**23**:625–7.
81. Peurala H, Greco D, Heikkinen T, *et al.* MiR-34a expression has an effect for lower risk of metastasis and associates with expression patterns predicting clinical outcome in breast cancer. *PLoS One* 2011;**6**:e26122.
82. Delfino KR, Rodriguez-Zas SL. Transcription factor-microRNA-target gene networks associated with ovarian cancer survival and recurrence. *PLoS One* 2013;**8**:e58608.
83. Cancer Genome Atlas Research N. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* 2008;**455**:1061–8.
84. Lang UE, Puls I, Muller DJ, *et al.* Molecular mechanisms of schizophrenia. *Cell Physiol Biochem* 2007;**20**:687–702.
85. Yamada K, Gerber DJ, Iwayama Y, *et al.* Genetic analysis of the calcineurin pathway identifies members of the EGR gene family, specifically EGR3, as potential susceptibility candidates in schizophrenia. *Proc Natl Acad Sci USA* 2007;**104**:2815–20.
86. Pittet JF, Griffiths MJ, Geiser T, *et al.* TGF-beta is a critical mediator of acute lung injury. *J Clin Invest* 2001;**107**:1537–44.
87. Cho JH, Gelinas R, Wang K, *et al.* Systems biology of interstitial lung diseases: integration of mRNA and microRNA expression changes. *BMC Med Genomics* 2011;**4**:8.
88. Helwak A, Kudla G, Dudnakova T, *et al.* Mapping the human miRNA interactome by CLASH reveals frequent noncanonical binding. *Cell* 2013;**153**:654–65.
89. Lee BK, Bhinge AA, Battenhouse A, *et al.* Cell-type specific and combinatorial usage of diverse transcription factors revealed by genome-wide binding studies in multiple human cells. *Genome Res* 2012;**22**:9–24.
90. Neph S, Stergachis AB, Reynolds A, *et al.* Circuitry and dynamics of human transcription factor regulatory networks. *Cell* 2012;**150**:1274–86.
91. Plaisier CL, Pan M, Baliga NS. A miRNA-regulatory network explains how dysregulated miRNAs perturb oncogenic processes across diverse cancers. *Genome Res* 2012;**22**:2302–14.