

Role of microRNAs in oncogenesis: Insights from computational and systems-level modeling approaches

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Abstract

MicroRNAs (miRNAs) are short (~22 nucleotides) non-coding RNAs that are often intricately integrated into the regulatory networks of various cellular processes and govern the cell fate decision making events associated with oncogenesis by regulating the gene expression through post-transcriptional modifications. miRNAs repress the target genes by either degrading the target mRNA or by inhibiting the process of translation. However, mathematical and computational modeling of miRNA-mediated target gene regulation in various cellular network motifs suggests that miRNAs play a much more complex role in cellular decision-making events. In this review, we give an overview of the quantitative insights obtained from such kind of mathematical modeling of miRNA mediated gene regulation by highlighting the various factors associated with miRNA regulation that are pivotal in diversifying the cell fate decisions related to oncogenesis. Intriguingly, recent experiments suggest that miRNAs can even upregulate the translation of the target protein under certain circumstances, which may lead to more complexities in miRNA-mediated gene regulations. We discussed possible avenues to explore such unusual biological observations related to miRNA's that can be modeled to get a detailed understanding of the influence of miRNAs in the context of oncogenesis.

Introduction

Micro RNAs (miRNAs) are endogenous, non-coding RNA molecules of very short length (~22 nucleotides), which are normally involved in gene silencing at the posttranscriptional level.¹⁻⁴ They bind to the target mRNA molecules with complementary sites and inhibit their target protein synthesis via two modes: (i) by degrading the target mRNA and (ii) by reducing the translation from the respective mRNA.^{2,5,6} MicroRNAs were first discovered in *C. elegans* by Lee and his colleagues⁷ in 1993 and now thousands of microRNAs have been identified in various species and deposited in online sequence depositories like miRbase database.^{6,8} A particular miRNA can target many mRNAs. Conversely, a single mRNA can be regulated by multiple miRNAs.^{9,10} Hence, several computational algorithms and methods have been developed to identify putative miRNA targets, which are later validated using experiments.¹¹ These studies unraveled that more than two-thirds of the human coding genes get regulated by miRNAs in different ways. Not only that, miRNAs confer robustness in noisy target gene expression,^{12,13} fine-tunes cellular decision-making events,¹⁴ and even upregulates target gene expression^{15,16} under specific circumstances. Thus, understanding miRNA mediated gene expression for a specific biological network or a process is a challenging task, due to this diverse nature of miRNA regulation.

Importantly, miRNAs are involved in several gene regulatory networks and control many cellular processes such as proliferation, differentiation, apoptosis, metabolism, invasiveness, etc., to influence cell-fate decisions.^{4,11,12,17,18} Several studies have shown that mutations or aberrant expression of miRNAs or components involved in miRNA biogenesis and regulation are associated with cancer development and progression.¹⁹⁻²² Thus, miRNAs are often found to be either oncogenic or tumor suppressors in a context-dependent manner.^{19,20,22,23} The abnormality in miRNA expression could arise due to gene amplification,²⁴⁻²⁶ deletion, or translocation²⁷⁻²⁹ of the genomic loci with miRNA genes. Even the miRNA

genes can be subjected to epigenetic modifications like protein-coding genes. In many cancers, epigenetic silencing of miRNA genes that may act as tumor suppressors has led to cancer.^{30–34} Studies have shown that epigenetic modification of miRNA genes could serve as potential biomarkers for cancer diagnosis and progression.^{30,33,34} This suggests that miRNA affects the proliferation response of cells in more than one way. Thus, the formation of a defective miRNA or a malfunctional miRNA can be potentially fatal for a living cell.

Dysregulation of enzymes involved in the miRNA biogenesis pathway (**Fig.1**) such as Dicer, Drosha, DGCR8, and Exportin, which are required for miRNA maturation and transportation can further lead to defective miRNA synthesis and localization.^{35–37} Once synthesized, miRNAs are loaded along with ribonucleoproteins like Ago to form a complex called RNA-induced silencing complex (RISC), which directs and facilitates the binding of miRNA to the 3'UTR binding site in the target mRNA. Any mutation in Ago proteins leads to impaired miRNA regulation.^{38,39} Another important alteration that can affect miRNA function is the presence of single nucleotide polymorphisms (SNPs), and mutations in the miRNA binding site of the mRNA.^{40,41} Such mutations can affect the miRNA: miRNA pairing and make the mRNAs insensitive to the miRNA^{19,42} On the contrary, certain mutations in 3'UTR of mRNAs can create new potential miRNA binding sites, which can increase the risk of uncontrolled proliferation. However, there are several instances in which the variation in 3'UTR by mutations/SNPs is found to be associated with reduced cancer risk.⁴²

Apart from these complex regulations, we must remember that miRNAs are often part of certain important gene regulatory motifs.^{18,43–46} Aberrant expression of miRNAs or their regulators affect the overall dynamical output of these motifs and lead to various cellular fates. However, these miRNA regulatory dynamics are highly complex and diverse. In this regard, mathematical and computational modeling studies^{47,48} proved quite insightful in describing the features of miRNA mediated gene regulation and their pivotal role in various gene regulatory motifs. One can envisage that studies combining quantitative experimental data along with mathematical or computational models could even lead to better insight into the miRNA mediated diverse gene expression responses, especially in the context of cellular proliferation. In this review, we gave a brief account of these kinds of theoretical and computational studies related to specific aspects of miRNA dynamics by highlighting the experimentally known facts about the miRNA regulations in general. We emphasized various experimental studies that dealt with the structural, dynamical, and concentration-dependent regulations of miRNA concerning the proliferation of mammalian cells, and discussed the role played by the mathematical or computational modeling in deciphering the comprehensive mechanistic insights about miRNA regulations. Finally, we concluded by showcasing the possible unexplored domains (such as miRNA mediated target gene upregulation via translation activation), where modeling studies can provide some initial insights to understand the complex and diverse miRNA regulation, especially in the direction of oncogenesis related issues.

MicroRNA mediated target repression

To begin with, it is important to get an overall understanding of how the miRNAs are synthesized and processed in mammalian cells. miRNAs are synthesized, processed, and assembled along with the Argonaute family of proteins (Ago 1- 4 in mammals) to form miRNA induced silencing (miRISC) complex.^{2,3,49}

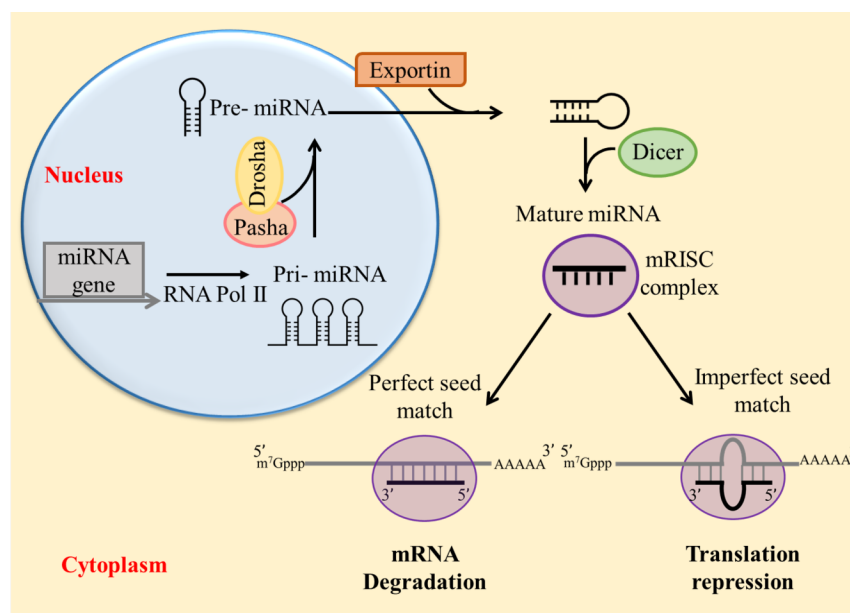


Fig. 1 miRNA biogenesis and mechanism of function . Pre-miRNAs are transcribed from the miRNA gene by RNA polymerase enzyme in the nucleus. They are processed by Drosha (RNase III enzyme) and Pasha (DGCR8) to give pre-miRNA transcripts of ~70 nucleotides length, which form stem-loop structures. Pre-miRNAs are exported to the cytoplasm, where they are processed by Dicer (RNase II enzyme) and then loaded into the miRISC complex containing the Argonaute (Ago) proteins. miRNA in the miRISC complex is guided by Ago towards the binding site in the target 3'UTR. After binding to the target mRNA, target repression occurs via mRNA degradation or translation based on the complementarity between the mRNA and miRNA seed sequence^{2,3,49}.

miRNAs in the miRISC complex then recognizes the target mRNA and directs it for decay and/or translation repression along with other RNA binding proteins (**Fig. 1**).^{2,3,49} The m⁷GTP cap structure in the 5' end and poly-A tail in the '3'end of mRNA is important to initiate translation in eukaryotes.⁵⁰ MicroRNAs are known to inhibit various stages of the translation process such as initiation, elongation, and maturation. The eukaryotic initiation factor complex consisting of eiF4F, eiF4G and eiF4A) recognizes and binds to the cap structure in the 5'end and recruits a small 40s ribosomal unit for translation. During translation, Poly A binding protein (PABP) recruited by GW182 binds to the poly-A tail present in the 3'end of mRNA and eiF4G interacts with PABP to circularize the mRNA.^{2,50} Binding of RISC complex with miRNA to the mRNA disrupts the interaction between eiF4F and PABP, causes decapping and deadenylation of the mRNA, which eventually leads to mRNA decay.^{2,51} Modeling these detailed events is not easy with limited quantitative experimental data. However, attempts have been made to model other features of miRNA regulation dealing with factors such as binding site complementarity, the number of binding sites, the relative abundance of miRNA and mRNA, presence of competing RNAs and RISC binding proteins, which influence the efficiency of miRNA mediated target repression effectively.⁵²⁻⁵⁵

Significance of the seed sequence in miRNA regulation

Usually, the nucleotides, 2-7nt in the 5' end of microRNAs have complementary nucleotide sequence, called seed match sequence in the 3'UTR of the target mRNA. Based on the degree of complementarity, the binding sites in the mRNA are classified as canonical (perfect match) and non-canonical sites (imperfect match).⁵⁵ While a perfect complementarity between miRNA and target seed sequence results in immediate mRNA decay, partial complementarity can lead to translation repression and/or mRNA degradation (**Fig. 2**). It is well-known that the extent of complementarity dictates the affinity for miRNA and mRNA interaction

and hence influences the miRNAs' inhibition efficacy.^{55–58} miRNAs inhibit mRNAs at an higher extent with a perfect seed matching, however, an imperfect match for seed sequence yields lower efficiency of miRNA mediated target mRNA degradation.⁵⁵ To investigate the role of seed sequence complementarity in miRNA's activity, Slutskin et al⁵² created a range of miRNA regulatory elements (MREs) of varying base-pair complementarity for ten miRNAs in K562 cells⁵² and reported similar observation as made by Agarwal et al.⁵⁵ Development of miRNA sequence databases and high throughput assays to measure miRNA and target abundance led to computational models that can accurately predict the extent of pairing and its effect on miRNA repression activity.^{55,56}

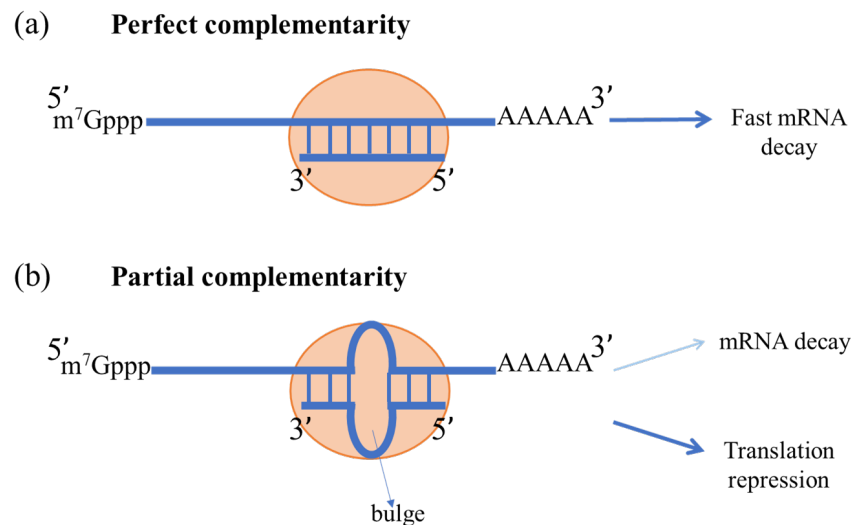


Fig. 2 Action of miRNA on a target based on seed sequence complementarity . (a) Perfect seed sequence match leads to fast mRNA degradation. (b) An imperfect match of miRNA with the seed sequence mainly causes translational repression.

These models suggest that the target sites with a match to 2-8 nt of miRNA (8-mer site), 7mer-m8 site, and 7mer site have a high affinity to interact with miRNA. Consequently, they have high miRNA activity.^{55,56} Reports have shown that the relative contribution and the order of two modes of inhibition (translation repression and mRNA decay) can vary in different organisms.^{59,60} Some sensitive miRNA studies suggest that majority of repression of target protein production is due to mRNA decay. In zebra fish embryos, the mRNAs with the short poly-A tail are more stable and for such mRNAs, translational repression efficiency is highly correlated with the Poly-A tail length.⁵⁹ A further kinetics-based study performed in *Drosophila* S2 and HeLa cells has revealed that gene silencing is achieved initially through translational repression and then followed by mRNA decay.^{60–62}

miRNA mediated repression depends on number of active binding sites and miRNA expression level

miRNAs are predominantly known to be modulators of the target gene expression. However, recent studies related to miRNA mediated target repression at the single-cell level indicate that they can even influence the target gene repression following a bi-stable switching mechanism. Under these circumstances, miRNA abundance and the number of MREs have a greater impact on the repression kinetics.^{53,54} Mukherji et al⁵³ used dual-color fluorescent reporters with and without binding sites with imperfect complementarity to miR-20a in the 3'UTR of the fluorescent genes. They have seen that there exists an mRNA threshold level below which the miRNA highly represses the corresponding mRNA. Once the mRNA level increases above the threshold, then mRNAs become free of the miRNA mediated repression. Mukherji et al⁵³ explained the

dynamics of microRNA-mediated switch-like repression kinetics using a molecular titration model (**Fig. 3**).⁵³ As the mRNA level increases, miRNA gets titrated due to the formation of miRNA:mRNA complex, and after a certain threshold, mRNA can escape the repressive effect. The model recreates the threshold behavior in miRNA-mediated repression observed in experiments. They further studied the fold repression by introducing a single binding site with perfect complementarity to miR-20a into the fluorescent gene. However, there was no mRNA threshold for gene expression in the presence of miR-20a. Instead, the fold repression gradually decreased with an increase in mRNA levels.

Next, they investigated the importance of various parameters such as miRNA level and rate of association between miRNA and mRNA associated with miRNA mediated target regulation. Increasing the association rate increased the sharpness of the threshold without affecting the threshold mRNA amount. However, altering the miRNA level influences both the sharpness of the threshold as well as the amount of mRNA accumulation required for escape from repression (**Fig. 3a**). Experimentally, to increase the binding affinity, the number of miR-20a binding sites in the 3'UTR were increased and miRNA levels were modulated using miR-20a mimics. They observed that increasing the binding sites sharpened the switch from full repression to escape from mir-20a mediated repression (**Fig. 3b**). However, the fold repression was higher at low protein levels for an increase in the binding sites, and there was no significant change at high protein levels. For four miR-20a binding sites in the 3'UTR, increasing the concentration of miRNA mimics sharpened the transition from repression to escape. The miRNA levels also modulated the target mRNA level required for the transition as predicted by the titration model.⁵³

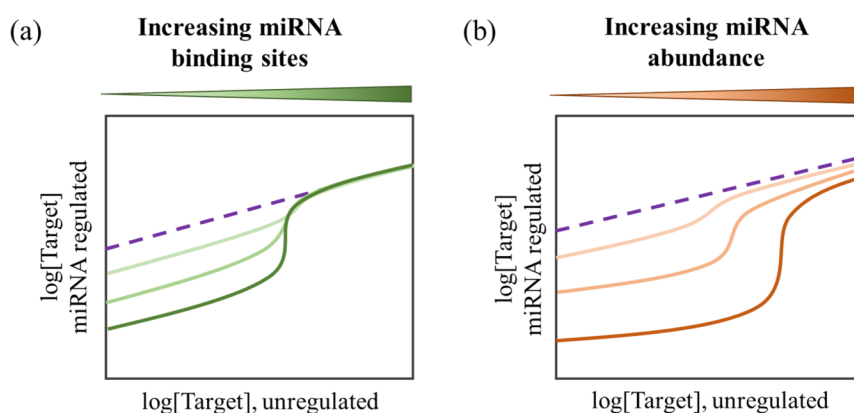


Fig. 3 Target threshold in relief from miRNA mediated repression using molecular titration model. The model assumes that the target mRNA can get produced and degraded intrinsically. After interacting with miRNA, mRNA gets degraded or dissociated from the complex, while the miRNA is recycled back into the system. The model also assumes that the protein translation can occur only from the free mRNA and the mRNA bound to the miRNA cannot undergo translation. Plots of target gene expression by varying, (a) the number of miRNA binding sites on the target, and (b) the concentration of the miRNA mimics for the same target with 'n' number of binding sites. Violet dotted lines represent the target expression in the absence of a miRNA binding site. Adapted from Mukherji et al⁵³.

Gam et al⁵⁴ investigated the fold repression using a similar dual fluorescence reporter but with a miRNA target site consisting of four perfectly complementary repeat sequences for the miRNA in the 3'UTR of the fluorescent gene.⁵⁴ They created a library of miRNA sensors for various miRNAs and observed the threshold kind of behavior similar to that of Mukherji et al⁵³ for a single miRNA binding site (four repeat sequences) with the perfect match. They found that there are three output regimes for miRNA mediated gene expression: (i) mRNA repressed regime (ii) mRNA derepressed regime and (iii) an in-between threshold regime (where switching from mRNA repression to de-repression takes place). They have demonstrated that the number

of binding sites and miRNA levels can shift the threshold for miRNA-mediated mRNA repression.⁵⁴ High throughput assays performed to measure miRNA activity for a large number of miRNAs in other cell lines further suggest that the factors such as miRNA abundance and no. of binding sites highly influence the miRNA repression activity.⁵²

Such sharp threshold response is observed for Notch (a signaling molecule) expression for color cancer stem cells (CCSCs), where mir-34a creates a bimodality in Notch expression by sequestering Notch mRNA.⁶³ The Notch pathway plays an important role in regulating asymmetric division in both normal and cancer stem cells, thus creating a CCSC daughter cell with self-renewal potential and a differentiated non-CCSC daughter cell. It has been observed that injecting these cells into the mouse xenograft models, low miR-34a expressing CCSCs promoted tumor growth due to symmetric division of CCSCs to give two CCSC daughter cells. On the other hand, high mir-34 expressing cells promoted differentiation with reduced tumorigenicity. Hence, the ability of microRNAs to fine-tune the symmetric and asymmetric division of cancer stem cells can be used as a potential strategy for cancer treatment.⁶³

miRNAs provide robustness in gene expression for a specific network motif

Gene regulatory networks controlling cell fate decisions consist of several feedback loops and redundant components to ensure robust cellular functioning even if one or few components fail^{18,45,64} to perform accordingly. In a mammalian cell, miRNAs are part of many Transcriptional Regulatory Networks (TRNs), and they function along with the transcription factors to regulate target gene expression via feedback or feedforward loops.^{17,43,44,65} It is well-known in the literature that gene transcription happens in a bursting manner⁶⁶⁻⁶⁹ and is a highly noisy process.^{66,69,70} The translation and degradation processes of proteins also involve fluctuations from the different origins within a cell. These perturbations can propagate through a regulatory network to generate high fluctuations in the mRNA and protein numbers.⁴⁶ Interestingly, for a feedforward loop motif (**Fig. 4a, right panel**), where an upstream protein activates miRNA and its target, any fluctuation in the upstream protein would drive miRNA and target expression in the same direction.⁷¹ In such networks, miRNAs act as noise buffers and fine-tune the steady-state protein levels to achieve uniform protein expression in the cell population.⁷¹

The noise in gene expression can arise from various intrinsic (inherent molecular fluctuations) or extrinsic (due to the cell-to-cell variabilities) sources.⁷² The stochasticity in gene expression is contributed from both the intrinsic as well as extrinsic sources.^{67,68,72,73} Using single-cell reporter assays and mathematical modeling, Schmiedel et al¹³ have revealed that in embryonic stem cells, miRNAs reduced the variability in protein expression for low expressing genes, while increasing the same for the highly expressed genes.¹³ They found that the combined regulation of genes by multiple miRNAs further reduced the protein expression noise.¹³ Studies along the same direction have indicated that miRNA in feedforward loops can contribute to a similar reduction in protein expression noise.⁷¹ Osella et al⁷⁴ created a stochastic model for a gene's expression, which is activated by a specific transcription factor (**Fig. 4a, left panel**).⁷⁴

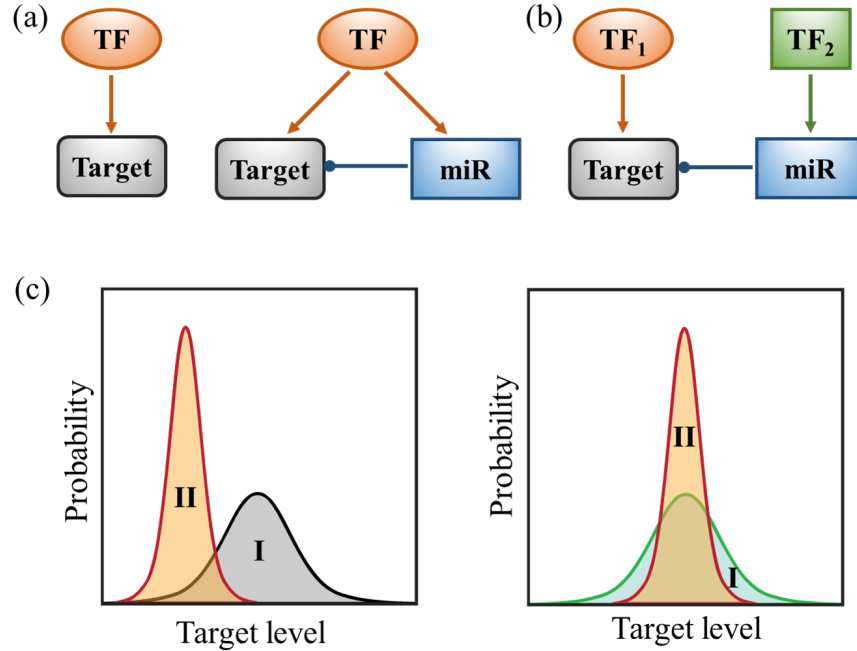


Fig. 4 miRNAs providing robustness in gene expression noise . (a) Simple target regulation by a transcription factor and an incoherent feed-forward loop involving miRNA. (b) The miRNA and its corresponding target are under the control of different TFs. (Solid arrows represent the activation process and round-headed arrows represent the inhibition process). (c) Target expression distribution in various network motifs presented in (a) and (b) , where introducing the miRNA regulation leads to suppression of noise (II) in comparison to the situation (I) when miRNA is absent or activated by different TFs. Adapted from Osella et al⁷⁴.

They created an incoherent FFL (**Fig. 4a, right panel**), where the transcription factor activates both the target gene and the miRNA that inhibits the target. miRNA being an extrinsic noise source is expected to increase the fluctuations in the gene expression. However, the probability distribution of protein expression level displays that miRNA regulation affects the mean of the distribution and reduces the coefficient of variation (**Fig. 4c, left panel (situation-II)**). They demonstrated that compared to open circuits (**Fig. 4b**) where target and miRNA are under the control of different TFs, an incoherent FFL (**Fig. 4c, right panel (situation-II)**) showed a lesser degree of fluctuations.⁷⁴ Such regulation is important especially in network motifs with positive feedbacks, where small perturbation in the signal, might drive the system to different protein steady states and affect the cellular fate decisions.

These theoretical studies are crucial to understanding networks where miRNAs are involved in TF-gene motifs that regulate cell fate decisions and any dysregulation in the corresponding motif components can lead to cancer. For example, miR-34a is a tumor suppressor miRNA and regulates several targets in cell proliferation, apoptosis, senescence such as MYCN, BCL2, SIRT1, E2F3, etc.⁷⁵ p53 is a transcription factor for mir-34a, and in several cancer types, mir-34a is found to be downregulated.⁷⁵ Another example is the network motif involving the protooncogene c-Myc, mir-17-92 (OncomiR), and their target transcription factor E2F in a feed-forward loop.^{14,76} Abnormality in the c-Myc, as well as mir-17-92 expressions, is linked with several cancer phenotypes.^{77,78}

miRNAs dictate the cell-fate decision making in cell cycle regulations

In the context of cell cycle regulation, the incoherent FFL of the Myc/E2F1/mir-17-92 network is known

to control cellular proliferation and explored rigorously via theoretical and computational modeling. Here, the cluster of miRNA (mir-17-92) precisely regulates the switching to different cellular states and avoids transitions due to small perturbations^{79,80} in the form of growth factor present in the cellular surroundings. E2F is a group of transcription factors (E2F1, E2F2, and E2F3) that activates many genes involved in cell cycle entry and progression, and its higher expressing state commonly indicate transition from G₁ to S phase. Interestingly, E2Fs are the target for miR-17-92 cluster components (**Fig. 5a**). The activation of E2F1 happens in a bistable manner (**Fig. 5a, right panel, orange line**), and switching from OFF to ON state happens when there is a sufficient amount of growth factor stimuli.⁸¹ In Myc/E2F1/mir-17-92 network (**Fig. 5b**), Myc activates the transcription of E2F and miR-17-92, when there are a growth factor signal and miR-17-92 components, in turn, inhibit E2F1.⁷⁶ E2F1 activates its transcription and also triggers Myc and miR-17-92 transcriptions. This leads to the formation of interconnected positive and negative feedback loops.⁷⁶

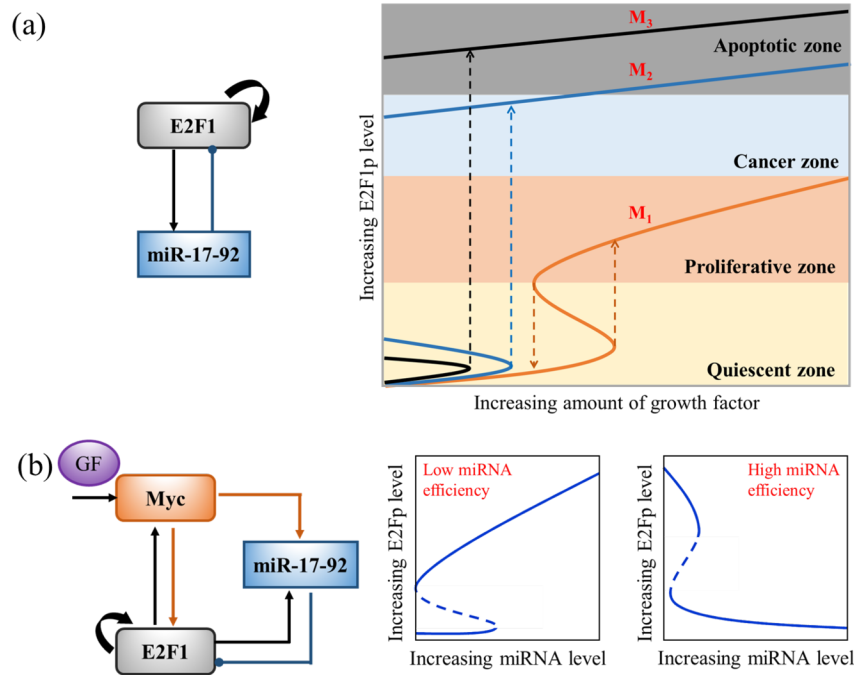


Fig. 5 miRNA (mir-17-92) governs the G₁-S transition by influencing bi-stable E2F1 dynamics.(a) E2F steady state dynamics as a function of growth factor level from a two-component network. M₁, M₂, and M₃ represent decreasing miRNA activity. Adapted from ⁸³. (b) Differential E2F steady state dynamics as a function of miRNA level for varying miRNA efficacy obtained using the Myc/E2F/miR-17-92 network. Adapted from Sengupta, Govindaraj, and Kar 2018.⁸⁴ (Solid arrows represent the activation process and round-headed arrows represent the inhibition process.)

Experimental studies have shown that the expression level of E2F1 during ON state, can drive the cell to any one of the states such as quiescence, proliferation, and apoptosis⁸² and miR-17-92 components fine-tune the E2F expression.^{79,80} Studies have shown that either overexpression or downregulation of miR-17-92 cluster components can lead to unwanted cell proliferation and cancer.⁷⁸ Aguda et al⁸³ proposed a model to explain these counterintuitive phenomena using a two-component network with E2F and miR-17-92 (**Fig. 5a**). Their model proposed that the expression level of mir-17-92 cluster components can modulate the growth factor threshold for E2F activation and 'ON' state E2F level (**Fig. 5a, right panel**), thus leading to different cellular states such as quiescence, proliferation, and apoptosis.⁸³ They suggest that the cells can enter into the cancer zone if the E2F 'ON' level lies between proliferation and apoptosis zone.⁸³ Thus,

upregulation of miRNA can allow the cells to escape apoptosis and become cancerous, and downregulation of miRNA can lead to unwanted proliferation.

Sengupta et al⁸⁴ proposed an alternative mechanism for differential miRNA regulation of E2F based on the miRNA efficiency.⁸⁴ They proposed a detailed model for Myc/E2F/mir-17-92 network (**Fig. 5b**) considering all possible positive and negative feedback interactions to explain the differential E2F regulation by miR-17-92. They predict that by modulating the miRNA related parameters such as miRNA mediated target degradation and translation repression rate, the model can be fine-tuned to give rise to two different scenarios as shown in **Fig. 5(b)** : (i) increase in E2F expression and (ii) decrease in E2F level, with an increase in miRNA level.⁸⁴ This explains why in certain solid cancers and some hematopoietic cancer types,^{78,85–99} the overexpression of mir-17-92 components leads to increased proliferation, however, the same causes suppression of proliferation for certain hematopoietic cancer types.^{78,100–102}

MicroRNAs in target gene upregulation

Even though we have a reasonable understanding of the miRNA regulatory networks, the diversity of miRNA mediated gene expression pattern never fails to surprise. Recent reports suggest that in certain conditions and specific cell types, miRNAs also upregulate target gene expression.^{15,103} Vasudevan et al¹⁰³ have observed that miR-369-3 upregulates the translation of reporter mRNA with AU-rich elements (ARE) in HeLa and HEK293T cells arrested in the quiescent/G0 phase.¹⁰³ During serum-starved conditions, microRNP protein known as FXR1 (fragile X mental retardation related protein 1) remains bounded with the AGO2 protein in the RISC complex. This leads to better base pairing between miR-369-3 and ARE, and results in translation upregulation.¹⁰³ Studies have shown that in liver cells, mir-122 enhances the translation of Hepatitis-C virus (HCV) RNA, which has IRES (internal ribosome entry site) within its 5'UTR. Mengardi et al⁵⁰ further revealed that binding of any miRNA can enhance the translation of the reporter RNA driven by HCV IRES by inserting let-7 and miR-451 binding sites in the 3'UTR of the HCV RNA.⁵⁰ Fascinatingly, in ribosomal protein (RP) mRNAs, miR-10a binding sites are present in their 5'UTR region downstream of the 5' TOP motif, which senses cellular stress and mitogen stimuli and controls RPs translation. Mir-10a enhances the translation of ribosomal proteins after binding to the 5'UTR and increases global protein synthesis, which has the potential for oncogenic transformation of cells.¹⁰⁴

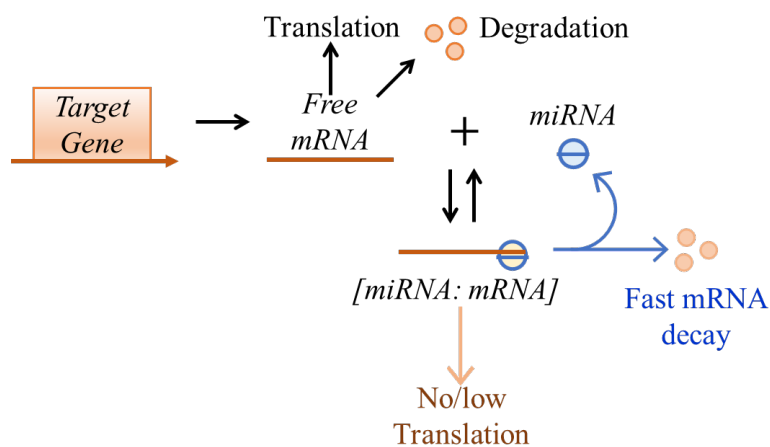
These experimental evidences suggest that miRNA can downregulate or upregulate target protein expression depending on the conditions and other given factors. One can envisage that the miRNA mediated target gene repression or upregulation mostly follow a similar mechanism (**Fig. 6**), but the dynamics of the overall process gets altered in a context-specific manner. In this regard, few mathematical models have been developed to explain what leads to such unexpected target protein upregulation by miRNA.^{84,105}

Sengupta et al⁸⁴ showed a similar upregulation in the expression of E2F1 by mir-17-92⁸⁴ using a detailed ODE-based mathematical model for Myc/E2F1/mir-17-92 network. In their model, miRNA forms complex with E2F1 mRNA, from which E2F1 mRNA gets degraded. They assumed that the translation can happen from the miRNA bounded E2F1 mRNA, but at a much lower rate than from the free mRNA. They have shown that depending on the degradation rate and the translation efficiency of E2F1 mRNA present in the complex, one can reproduce a scenario where the corresponding miRNA can either repress to upregulate the E2F1 protein expression (**Fig. 6**).⁸⁴

Nyananiti and Gadgil provided a plausible explanation for such target upregulation by using a mathematical model in which multiple miRNAs compete for a single mRNA.¹⁰⁵ They created a detailed model for a single mRNA regulated by two miRNAs by considering all the biochemical processes such as transcription, translation, and degradation. In their model, mRNA can form complex with any one of the two available miRNAs and also with both the miRNAs, if the binding sites are not overlapping. From the miRNA: mRNA complex, either the target or the miRNA can get degraded while the other counterpart gets recycled back. All the species in the model has inherent degradation rates. They also assumed that protein translation can happen from both free and miRNA bound mRNA. From simulations, they have observed that an increase in miRNA level always results in decreased protein levels when a single miRNA targets the mRNA. However,

when there is combinatorial regulation by two miRNAs and there is an overlapping of the two MREs, such that two miRNAs cannot bind at the same time, they observe an unexpected positive effect of miRNA on target protein abundance in certain parameter space.

(a) **Target repression**



(b) **Target upregulation**

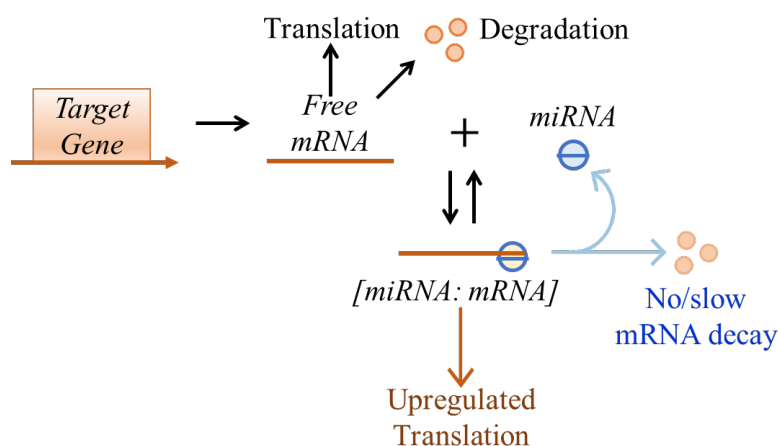


Fig. 6 Target regulation by microRNAs (as discussed by Sengupta et al⁸⁴). (a) After binding to the mRNA, miRNA immediately activates the target mRNA degradation and causes target repression. (b) Low target degradation after miRNA binding and translation of miRNA bound complex can result in target upregulation.

They predicted that the stabilization of the target mRNA due to miRNA binding and translation of protein from miRNA bound mRNA are important to achieve the target protein upregulation by the miRNA.¹⁰⁵ The above-mentioned studies suggest that translation upregulation is possible when the miRNA:mRNA complex is somehow stabilized and the degradation of mRNA along with the translation inhibition is not high from the corresponding complex (**Fig. 6b**). It was further suggested by certain studies that the presence of the regulatory sequences such as ARE binding motifs, poly C motifs can facilitate the binding of RNA binding proteins, which stabilizes mRNAs and upregulates translation.^{106,107}

Competing endogenous RNAs in microRNA regulation

As described in the previous section, a single mRNA can contain binding sites for multiple miRNAs. Similarly, a single miRNA can target multiple mRNAs. However, a set of non-coding transcripts that possess miRNA response elements (MREs) similar to target mRNA, bind to the miRNAs and prevent them from acting on target mRNA.¹⁰⁸ These non-coding transcripts control the amount of miRNA available for target mRNA, thereby indirectly modulating the target protein expression. RNA transcripts that share a binding site for the same miRNA and regulates each other's expression by competing for that miRNA are called competing RNAs or ceRNAs. These ceRNAs are present in various forms such as long coding RNAs, circular RNAs, pseudogenes or they could also be protein-coding mRNAs.¹⁰ CeRNAs provides an additional layer of complexity to the miRNA mediated gene regulation. Experimental studies indicate that they have major implications in diseases like cancer.^{109–111} For example, PTENP1 is a pseudogene for PTEN and they share similar MREs in the 3'UTR region. PTENP1 captures miRNAs targeting PTEN and helps in its function as a tumor suppressor. PTENP1 is found to be deleted in various cancers and overexpression of PTENP1 resulted in increased PTEN expression and induced growth inhibition by sequestering the miRNAs.^{111–113} Several non-coding transcripts that regulate PTEN are found to be expressed in a correlated manner in colon cancer cells, where PTEN expression or copy number is altered.¹¹²

Philip et al¹¹⁴ created artificial miRNA sponges with multiple binding sites for miR-20a and found that the addition of miR-20a sponges significantly alters the miR-20a target gene expression.¹¹⁴ Studies also suggest that miRNA activity does not depend on its levels, but the relative abundance of target sites can appreciably influence its activity.^{9,115–117} However, the ceRNA hypothesis remains controversial because miRNA target sites are usually present in abundance, and alteration in the level of one target mRNA might not considerably affect other miRNA target's expression level.^{115,116} Boson et al¹¹⁷ quantified the concentration of endogenous target and miRNAs using biochemical techniques such as mRNA-seq, small RNA- seq, and iCLIP in mouse cell lines to calculate miRNA: target ratio. They essentially investigated how modulating the ratio using ceRNAs of varying affinity affects miRNA binding.¹¹⁷ They have shown that ceRNAs with high affinity than the target can derepress the miRNA targets only for miRNA families with low miRNA: target ratio. If the miRNA: target ratio is high or the ceRNA affinity is low, then there is no noteworthy effect of ceRNA regulation on the target. They proposed a simple target competition model with hierarchical affinity for targets, which predicts that miRNA: target ratio determines the susceptibility to ceRNA competition at identical level of high affinity ceRNA overexpression.¹¹⁷ However, this aspect of miRNA regulation has to be investigated in a much-detailed manner using theoretical or computational modeling.

Conclusion

The diversity of miRNA activity in the cellular system is noteworthy, and hard to comprehend by just performing high-quality experiments. In recent years, investigations using combined high throughput experimental techniques and computational methods for finding miRNA targets and miRNA activity provided a detailed understanding of miRNA-mediated gene regulation.^{52–56} However, in gene regulatory networks, where miRNAs themselves are regulated by other proteins and are part of complex dynamics, network-specific analyses are required. For example; multiple mRNAs compete for a miRNA, or multiple miRNAs targeting a single mRNA. It is worthwhile to look into the target repression dynamics when multiple miRNAs of varying inhibition efficiency act on the same target. A recent study in *C. elegans* for Lin 4 protein show that the location of binding sites can favor different modes of inhibition like mRNA decay when miRNA binds to 3'UTR and translation repression for 5'UTR binding.¹¹⁸ Single-cell studies have provided more insights into the dynamics of miRNA repression kinetics and turned out to be the major determinants of miRNA activity.^{53,54} miRNAs also upregulate target expression in the presence of certain RNA binding proteins and environment conditions.^{15,106,107} Even though few models have proposed a possible mechanism for miRNA mediated target upregulation,^{84,105} their predictions have to be validated by experiments.

More computational algorithms and mathematical models have to be developed to investigate the interaction between RNA binding proteins and miRNAs and how such interactions affect the miRNA function. Especially in the context of noise buffering by miRNAs, understanding complex networks involving miRNAs and the

effect of competing RNA's in miRNA regulation, mathematical and computational models will play a critical role in understanding the miRNA dynamics. A system biology approach will be crucial to decipher the actual mode of action of miRNA in a network-specific manner. Getting such a quantitative understanding of miRNA-mediated gene regulation in signaling pathways involved in cancer and other related diseases will enable us to develop novel therapeutic strategies for the same.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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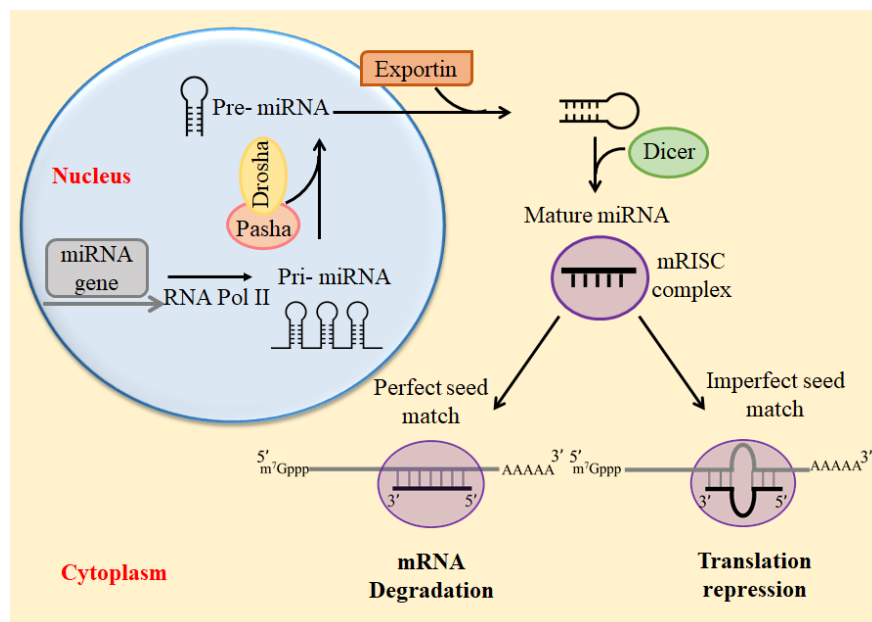
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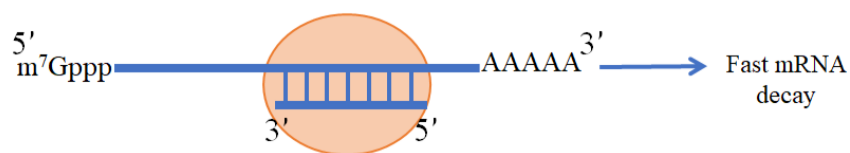
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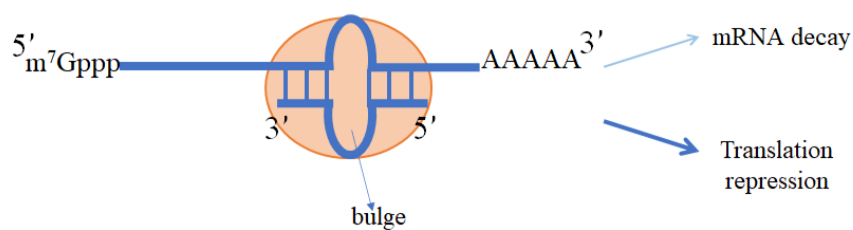
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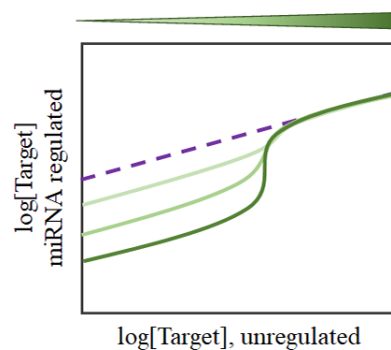
(a) **Perfect complementarity**



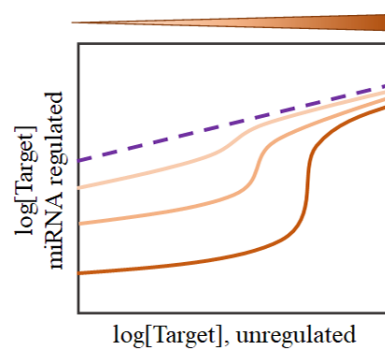
(b) **Partial complementarity**

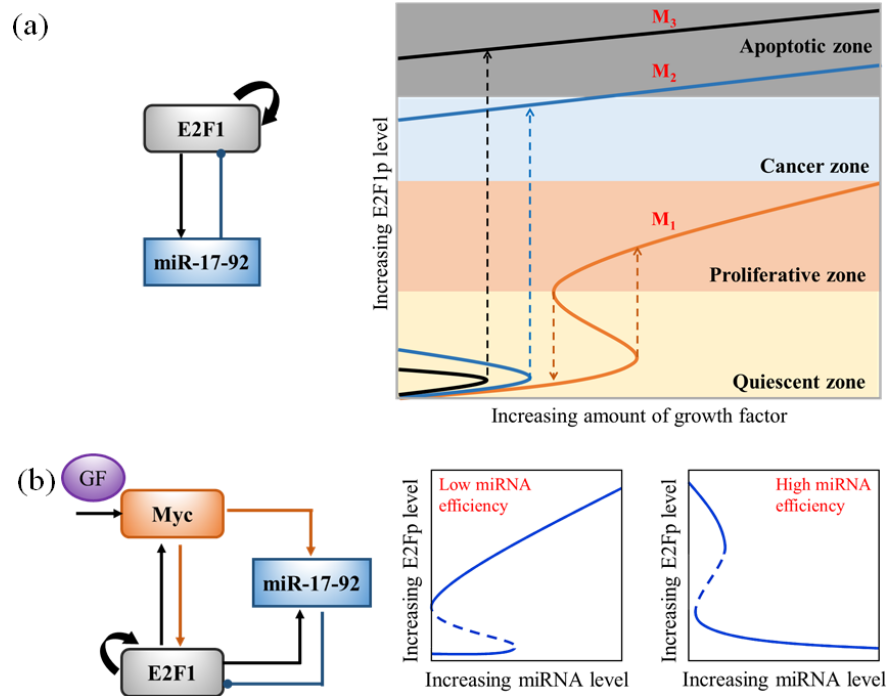
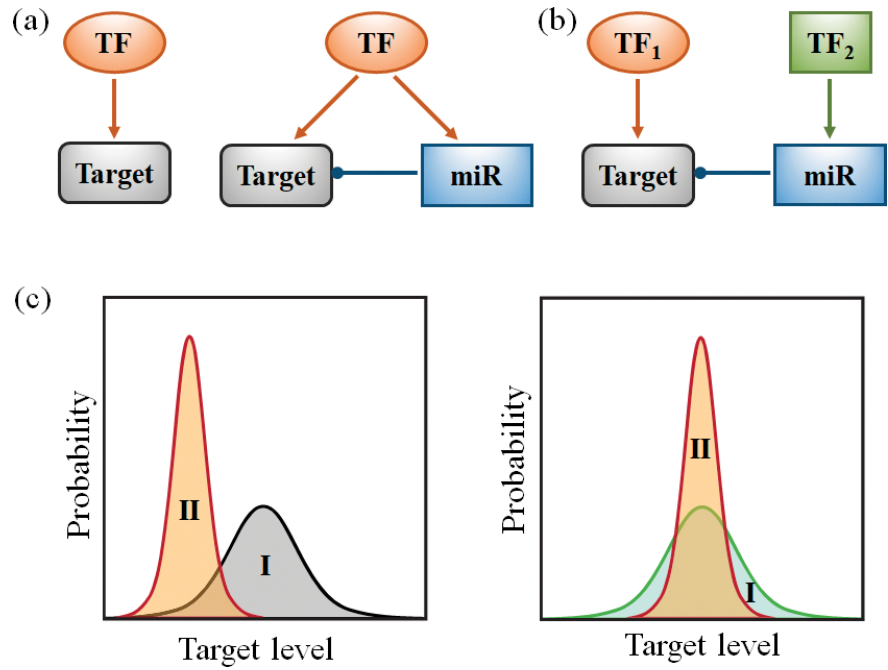


(a) **Increasing miRNA binding sites**

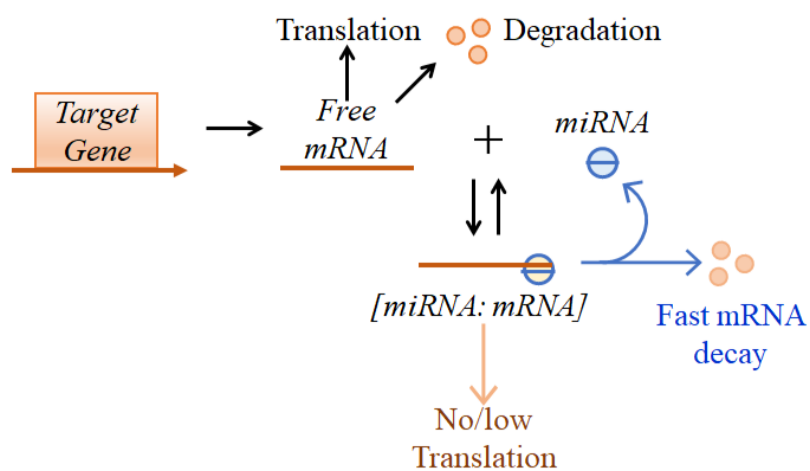


(b) **Increasing miRNA abundance**





(a) **Target repression**



(b) **Target upregulation**

