Deciphering the rules of ceRNA networks

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The Flip Side of miRNA Function

Recent theoretical and experimental studies have shed light on the complex network of interactions among the multiple classes of RNA within the cell. Although much of the focus over the past decade has been on the mechanisms by which microRNAs (miRNA) regulate the stability and translation of messenger RNAs (mRNAs), more recently it has come to light that the mRNA targets themselves are not merely passive substrates for miRNA repression but key elements in regulating miRNA availability within cells (1). This reverse logic provides a unique mode of miRNA regulation, alongside the already characterized transcriptional and posttranscriptional roles (2, 3), and compels a redefinition of the rules governing miRNA circuitry. In PNAS, Ala et al. (4) report a mathematical model for the qualitative dissection of interactions among the diverse classes of cellular RNAs, as well as experimental validation, thereby providing a basis for defining and describing complex RNA-based regulatory networks.

The first proof-of-principle that cellular miRNA abundance could be titrated for regulatory effect emerged from studies of artificial transcripts containing tandem repeats of miRNA responsive elements (MREs), called "miRNA-sponges" (5, 6). Acting via stoichiometric interactions and by principles of mass action, MREs become an RNA-based regulatory mechanism for modulating miRNA action. Evidence from both mammalian and plant systems supported the existence of endogenous mechanisms of miRNA titration, whereby mRNAs, pseudogenes and long noncoding RNAs compete for miRNA binding (7–9). Very recently, the repertoire of "competing endogenous RNA" (ceRNA) has been expanded by the identification of a new class of circular RNA (10, 11). Compared with pseudogenes, long noncoding RNAs and circular RNAs, the effects of the decoy activity of a protein-coding mRNA is most profound. Indeed,

the binding of a miRNA to the 3'UTR of a target mRNA affects not just the abundance of its immediate protein target, but by relieving other potential mRNA targets from similar repression, can influence the abundance of a network of other proteins. Thus, an approach to modeling the ceRNA

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system is becoming a prerogative for a clear comprehension of this unique regulatory role of RNA networks.

Modeling ceRNA Cross-Talk in the Cell

Ala et al. (4) define a precise set of rules to illuminate the "communication" within the ceRNA networks and to illustrate how perturbations of different system components affect overall network equilibrium. The kinetic model proposed relies on a titration mechanism that, by establishing a threshold level of effect, orchestrates the interactions within the ceRNA network. Based on a simple set of interactions among one miRNA and two target mRNAs (ceRNAs), the authors hypothesized that a near equimolar equilibrium of all of the elements is required for optimal ceRNA-mediated cross-regulation. Transcription and degradation rates for both the miRNA and the ceRNAs, and the association, dissociation, and degradation rates for the miRNA/ceRNA complexes represent the key parameters of the kinetic model.

Another key element highlighted by the analysis of Ala et al. (4) is the effect of RNA dosage. Indeed, the basal expression levels of the different components of an RNA network appears to be crucial for modulating the output of the system. The relevance of ceRNA dosage was validated by the authors in experiments with phosphatase and tensin homolog (PTEN), its ceRNA vesicle-associated membrane protein-associated protein A (VAPA), and eight miRNAs shown to target both of them. By evaluating a series of cell lines with variable ratios of VAPA:PTEN but similar combined levels of miRNAs, and then perturbing the system by introducing siRNA against VAPA, the authors showed that silencing the higher expressed ceRNA (VAPA) provided a stronger effect on the lower expressed one (PTEN) when the two species were expressed at nearly equimolar concentrations. On the other hand, silencing VAPA in a context of similar VAPA:PTEN ratio but with increased miRNA expression decreased PTEN expression proportionally to the amount of miRNA expression. These in vitro studies confirmed the predictions of the theoretical model, supporting the notion that ceRNA dosage is critical for ceRNA activity. In other validation studies, the authors show that increasing the number of shared miRNAs intensifies the interactions within a ceRNA network (ceRNETS).

As with canonical gene-expression regulation, the authors postulated that indirect interactions in ceRNETS amplify the effect of perturbations of the different components of the system. The authors built an unbiased network of predicted ceRNA interactions by integrating MRE levels and conserved co-expression in some 7,500 annotated samples. By Gene Ontology analysis, the authors discovered an overrepresentation of genes involved in transcription and the regulation of gene expression, thereby implicating cross-regulation of transcription factors and ceRNETS, as well as genes involved in miRNA processing [e.g., Dicer and argonaute RISC catalytic component 1 (AGO1)], suggesting

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that ceRNETs play a role in regulating the miRNA processing machinery.

To provide experimental validation of the interactions suggested by their model, the authors analyzed the network involving the oncogenic PAX/FKHR translocation [commonly involving paired box (PAX)3 or PAX7 and forkhead box O1 (FOXO1) in 90% of alveolar rhabdomyosarcoma (ARMS)], its ceRNAs, and the ceRNAs of its transcriptional targets implicated in ARMS. The PAX/FKHR translocation disrupts the FOXO1 transcription factor, leading to transcriptional up-regulation of MET, fibroblast growth factor receptor 4, insulin-like growth factor 1 receptor, and anaplastic lymphoma receptor tyrosine kinase (ALK) in ARMS with respect to embryonal rhabdomyosarcoma (ERMS). Analysis of ceRNAs of FOXO1 and its transcriptional targets predicted increased levels in ARMS. The authors validated this network by ectopically overexpressing the PAX/FKHR fusion transcript in an ERMS cell line, and noting an increase in the expression of some ALK and MET ceRNAs. By interrogating the ceRNET for FOXO1 transcriptional targets, Ala et al. (4) find that over 40% of genes up-regulated in ARMS compared with ERMS were ceRNAs of FOXO1 targets. The cross-regulation of transcription factors and the ceRNET was further demonstrated by modulating miRNA expression and observing the effects on transcription factor ceRNAs. Finally, by calculating the Pearson coefficient of coexpression between the transcription factors and their ceRNAs or transcriptional

targets, in the setting of different miRNA expression levels, Ala et al. confirmed that ceRNA-mediated cross-regulation depends on miRNA fluctuations, and the activity of transcription factors toward their targets is more stable and less dependent on miRNA fluctuations.

As originally proposed by Salmena et al. (12), ceRNAs represent a hidden RNA “language”: a network of interactions of various RNA species that coordinately regulate gene expression. By integrating mathematical modeling, informatics, and experimental validation, the Ala et al. study (4) provides a significant advance in describing these complex networks in the cell and predicting outcomes of perturbations to the networks. The observation that transcription factors are intimately interconnected with ceRNA networks, and that indirect ceRNA interactions amplify their influence on gene expression, appoints ceRNAs as key

players in gene regulation. In light of this observation, ceRNAs likely act as additional molecular drivers of evolution, as already demonstrated for miRNA genes (13). No doubt, the extensive miRNA-mediated interactions highlighted by the authors will provide important clues to understanding the regulation of normal cell physiology and its deregulation in pathologic states like cancer.

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