MicroRNAs as a Selective Channel of Communication between Competing RNAs: a Steady-State Theory

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SUPPORTING MATERIAL

MicroRNAs as a selective channel of communication between competing RNAs: a steady-state theory

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Derivation of miRNA steady-state concentration

An approximate, explicit expression for the steady state miRNA level can be derived starting from (9) of the Main Text. For simplicity, let us consider the case of $N = 2$ ceRNAs, in which (8) of the Main Text reduces to the equation

$$[\mu]\left[\delta + b_1 z_1 F_1([\mu]) + b_2 z_2 F_2([\mu])\right] = \beta \ . \tag{1}$$

We can work out its solutions explicitly depending on the regimes to which the ceRNAs belong by inserting (9) of the Main Text into (1), using the relation $w_i = z_i \mu_{0,i}$ and keeping only linear terms in $[\mu]$. One finds the following results:

(a) $m_1, m_2 \in \mathcal{F}$:

$$[\mu] \simeq \frac{\beta}{\delta + \sum_i b_i z_i} \tag{2}$$

(b) $m_1, m_2 \in \mathcal{S}$:

$$[\mu] \simeq \frac{\beta - \frac{1}{4}\sum_i b_i w_i}{\delta + \frac{1}{4}\sum_i b_i z_i} \tag{3}$$

(c) $m_1, m_2 \in \mathcal{B}$:

$$[\mu] \simeq \frac{\beta - \sum_i b_i w_i}{\delta} \tag{4}$$

(d) $m_1 \in \mathcal{B}$, $m_2 \in \mathcal{S}$:

$$[\mu] \simeq \frac{\beta - b_1 w_1 - \frac{1}{4} b_2 w_2}{\delta + \frac{1}{4} b_2 z_2} \tag{5}$$

(e) $m_1 \in \mathcal{F}$, $m_2 \in \mathcal{B}$:

$$[\mu] \simeq \frac{\beta - b_2 w_2}{\delta + b_1 z_1} \tag{6}$$

(f) $m_1 \in \mathcal{F}$, $m_2 \in \mathcal{S}$:

$$[\mu] \simeq \frac{\beta - \frac{1}{4} b_2 w_2}{\delta + b_1 z_1 + \frac{1}{4} b_2 z_2} \tag{7}$$

Extending to the general case of N ceRNAs we conclude that

$$[\mu] \simeq \frac{\beta - \sum_{i \in \mathcal{B}} b_i w_i - \frac{1}{4} \sum_{i \in \mathcal{S}} b_i w_i}{\delta + \sum_{i \in \mathcal{F}} b_i z_i + \frac{1}{4} \sum_{i \in \mathcal{S}} b_i z_i} \ , \tag{8}$$

The mirror system: one target, M miRNA species

The dual system in which M miRNA species, labeled μ_α ($\alpha = 1, \ldots, M$), target the same RNA m (to avoid confusion we will keep referring to it as a ceRNA even though in this case it is not really competing, being the only target species), can be worked out in full analogy with the case discussed above. In particular, defining $\mu_\alpha^\star = \beta_\alpha / \delta_\alpha$, we have that, at stationarity, the level of free miRNA species is given by

$$[\mu_\alpha] = \mu_\alpha^\star F_\alpha([m]) \ , \tag{9}$$

where

$$F_\alpha = \frac{m_{0,\alpha}}{[m] + m_{0,\alpha}} \quad , \quad m_{0,\alpha} = \frac{\delta_\alpha}{k_\alpha^+}(1 + \psi_\alpha) \tag{10}$$

with $\psi_\alpha = (k_\alpha^- + \kappa_\alpha)/\sigma_\alpha$. (Note that now rates carry the index of the corresponding miRNA involved.) The free ceRNA level on the other hand results from the algebraic equation

$$[m] \left[d + \sum_\alpha \beta_\alpha \zeta_\alpha F_\alpha([m]) \right] = b \ , \tag{11}$$

where $\zeta_\alpha = (\sigma_\alpha + \kappa_\alpha)/(m_{0,\alpha}\sigma_\alpha)$. As before, each $m_{0,\alpha}$ can be interpreted as reference level for the target which can be used to separate different regimes for the miRNA species. Borrowing the terminology used in the previous case, we have

$$F_\alpha([m]) \simeq \begin{cases} 1 - [m]/m_{0\alpha} & \alpha \in \mathcal{F} \\ \frac{1}{2} - ([m] - m_{0,\alpha})/(4m_{0,\alpha}) & \alpha \in \mathcal{S} \\ m_{0,\alpha}/[m] & \alpha \in \mathcal{B} \end{cases} \tag{12}$$

where $[m] \ll m_{0,\alpha}$ for a 'free' miRNA, $[m] \simeq m_{0,\alpha}$ for a 'susceptible' miRNA, and $[m] \gg m_{0,\alpha}$ for a 'bound miRNA. In turn, the level of free ceRNA is given by

$$[m] \simeq \frac{b - \sum_{\alpha\in\mathcal{B}} \beta_\alpha \omega_\alpha - \frac{1}{4}\sum_{\alpha\in\mathcal{S}} \beta_\alpha \omega_\alpha}{d + \sum_{\alpha\in\mathcal{F}} \beta_\alpha \zeta_\alpha + \frac{1}{4}\sum_{\alpha\in\mathcal{S}} \beta_\alpha \zeta_\alpha} \tag{13}$$

where $\omega_\alpha = \zeta_\alpha\, m_{0,\alpha} = (\sigma_\alpha + \kappa_\alpha)/\sigma_\alpha$, while for the susceptibility we obtain

$$\chi_{\alpha\gamma} \equiv \frac{\partial[\mu_\alpha]}{\partial\beta_\gamma} = \frac{1}{\delta_\alpha}\left[F_\alpha([m])\delta_{\alpha\gamma} + \frac{\beta_\alpha\zeta_\gamma\chi_{m,m}}{4[m]} W_{R(\alpha),R(\gamma)}\right] \tag{14}$$

where $\chi_{m,m} = \frac{\partial[m]}{\partial b}$ and the matrix $\widehat{W}$ is the same as in (14) of the Main Text with $[m]$, $m_{0,\alpha}$ and $m_{0,\gamma}$ replacing respectively $[\mu]$, $\mu_{0,i}$ and $\mu_{0,j}$.

Therefore the cross-talk that is established between miRNAs is, as before, selectively turned on only for species lying in particular regimes, defined by the free ceRNA level. In complete analogy to the dual system analyzed in the main text, two types of effective interactions arise: the first one is symmetric encodes the response of a miRNA in the $\mathcal{S}$-regime to a perturbation of another miRNA in the $\mathcal{S}$-regime; the second one is asymmetric and encodes the response of a miRNA in the $\mathcal{S}$-regime to a perturbation of a miRNA in the $\mathcal{B}$-regime. An example of a pattern of interactions between miRNAs is shown in Figure 1. Note that the intensity of the cross-talk is modulated by the factor ζ_γ and increases when the rate of catalytic degradation increases. If $\sigma_\alpha \to 0$ for all α implies $m_{0,\alpha} \to \infty$: in this case, all miRNA species lie in the $\mathcal{F}$-regime and no cross-talk is possible at steady state.

The role of topology

Network topology can play an important role as a cross-talk enhancer. In specific, we will now argue that ceRNA-ceRNA interactions can be mediated by a large number of miRNA species which individually would only weakly dampen ceRNA

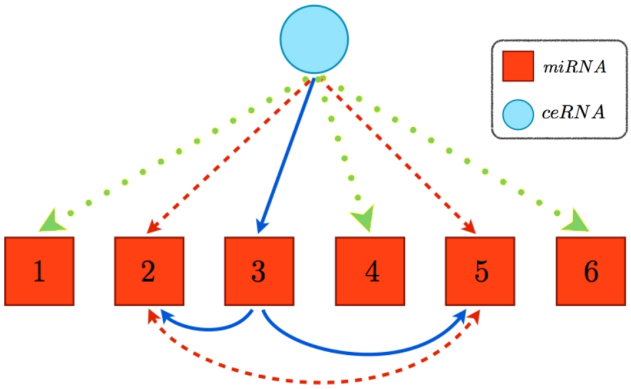

Figure 1: Schematic representation of a system of one target RNA and $M = 6$ miRNAs species. In this case miRNA 3 is Bound, miRNAs 2 and 5 are Susceptible and the remaining are Free from the target RNA. Cross-talk interactions pattern is derived analogously to the dual case discussed in the main text: symmetrical cross-talk interactions emerge between miRNA 2 and 5 and asymmetrical interactions emerge from miRNA 3 to miRNAs 2 and 5.

levels. We consider a diluted network described by an adjacency matrix $\{A_{i\alpha}\}$ such that

$$A_{i\alpha} = \begin{cases} 1 & \text{if ceRNA } m_i \text{ is targeted by miRNA } \mu_\alpha \\ 0 & \text{otherwise} \end{cases} \tag{15}$$

making the following simplifying assumptions: (a) the network is kinetically homogeneous, i.e. rates are the same for all ceRNAs, so that $\mu_{0,i\alpha} = \mu_0$ for each i and α and $b_i = b$, $d_i = d$ for each i; (b) miRNA levels are uniform, i.e. $[\mu_\alpha] = [\mu]$ for all α; (c) $[\mu]/\mu_0 \equiv t \ll 1$, so that all ceRNA species are in the $\mathcal{F}$-regime with respect to any miRNA ($i \in \mathcal{F}(\alpha)\ \forall i, \alpha$).

Consider a pair of ceRNAs m_i and m_j, targeted respectively by $n_i = \sum_\alpha A_{i\alpha}$ and $n_j = \sum_\alpha A_{j\alpha}$ miRNA species, $n_{ij} = \sum_\alpha A_{i\alpha}A_{j\alpha}$ of which are in common. In this case, the ceRNA concentration reads $m_i = m^\star/(1 + n_i t)$ (with $m^\star = b/d$) and the cross-susceptibility (28) of the Main Text turns out to be given by

$$\chi_{ij,\alpha} = \begin{cases} \frac{1}{dK_\alpha} \frac{t}{[1+t(n_j-1)][1+t(n_i-1)]^2} & \text{if} \quad A_{i\alpha}A_{j\alpha} = 1 \\ 0 & \textit{otherwise} \end{cases} \tag{16}$$

where $K_\alpha \simeq [\delta/(zb) + \sum_{k\in\alpha}(1 + tn_k)^{-1}]$ and z is defined by the fact that $z_{i\alpha} = \frac{\sigma}{(\sigma+\kappa)\mu_0} = z$ for each i and α. (The notation $k \in \alpha$ indicates all ceRNAs interacting

with miRNA μ_α.) As expected, the dilution increases upon increasing the number of ceRNAs interacting with a given miRNA species μ_α (each of them add a positive term $(1 + tn_k)^{-1}$ to K_α thus making it larger) and upon increasing n_i and n_j, since

$$\chi_{ij}^{\alpha} \propto \frac{1}{n_j n_i^2} \qquad (n_i, n_j \gg 1/t) \ . \tag{17}$$

Consider now the particular case of a regular bipartite network with fixed ceRNA and miRNA connectivity so that $n_i = n$ for each i and $\nu_\alpha \equiv \sum_i A_{i\alpha} = \nu$ for each α. Setting

$$K_\alpha = K = \frac{\delta}{zb} + \frac{\nu}{1 + tn} \tag{18}$$

for all α we clearly see that now each miRNA species contributes equally to the overall susceptibility, i.e, $\chi_{ij,\alpha} = \chi_0$ for all i and j targeted by μ_α with

$$\chi_0 = \frac{1}{dK} \frac{t}{[1 + t(n-1)][1 + t(n-1)]^2} \ , \tag{19}$$

while the overall susceptibility is given by $\chi_{ij} = n_{ij}\chi_0$. The contribution of a single miRNA to the overall susceptibilities will depend on the value of t. In particular, one easily sees that

$$\chi_0 = \begin{cases} \frac{t}{dK} \sim O(\frac{\epsilon}{n}) & \text{for } t \ll 1/n \\ \frac{1}{dKn} \sim O(\frac{1}{n}) & \text{for } t \simeq 1/n \\ \frac{1}{dKt^2n^3} \sim O(\frac{\epsilon}{n}) & \text{for } t \gg 1/n \end{cases} \tag{20}$$

Generalizing the Free, Susceptible and Bound regimes, one realizes that the case $t \ll 1/n$ (resp. $t \simeq 1/n$ and $t \gg 1/n$) describes a ceRNA that is 'globally free' (resp. 'globally susceptible' and 'globally bound') with respect to the overall miRNA population. We therefore conclude that χ_{ij}

(i) increases with the number n_{ij} of miRNA species shared by the ceRNAs m_i and m_j;

(ii) decreases if the shared miRNAs have many other targets;

(iii) peaks when ceRNAs are 'globally susceptible' to the overall miRNA population, and it can be of the same order of magnitude as the self-susceptibility, i.e. $O(1/d)$, when $n_{ij} \simeq n$.

Perhaps most remarkably, the cross-talk can be effective even among ceRNAs that are in the Free regime with respect to individual miRNAs, provided they are commonly targeted by a large number of miRNA species thus becoming 'globally susceptible'. However, in order to achieve efficient cross-talk strong correlations in the network connectivity are needed (large n_{ij}): highly clustered networks can allow for much stronger cross-talk than random graphs (see Figure 2).

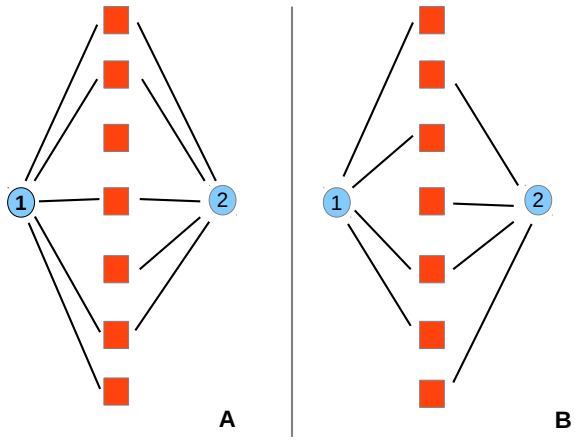

Figure 2: Two examples of different network structures with $N = 2$ ceRNAs (blue circles) and $M = 7$ miRNAs (red squares). A) A highly correlated network structure where ceRNAs share almost all of their regulators ($n_1 = n_2 = 5,\ n_{12} = 4$). B) A poorly correlated structure where ceRNAs share a small fraction of their regulators ($n_1 = n_2 = 4,\ n_{12} = 1$). Cross-talk will tipically be much stronger in A than in B.

The miRNA-decoy transcript

Many miRNAs (possibly about 50% of the total [1]) are hosted in non-coding genes whose transcript can incur a dual fate: after transcription, the precursors can either be processed into mature miRNAs through a series of steps involving proteins DROSHA and DICER, or they can reach the cytoplasm unprocessed in the form of long non-coding RNAs (lncRNAs). The RNA sequence close to the sites corresponding to the miRNA presents a region with a sequence that is almost complementary to that of miRNA. These proximal strings allow for the miRNA precursor (pri-miRNA) to take on the peculiar hairpin structure that is essential for the recognition by the processing proteins and thus for miRNA maturation [2]. It also follows, however, that the RNA sequence close to the miRNA necessarily contains a good potential binding site for the miRNA itself. When matured into lncRNAs, such transcripts are thus targeted by the miRNA and represent efficient 'miRNA traps' or decoys, through which the population of miRNAs available for target repression can be regulated. The above miRNA-decoy mechanism can be modeled with following processes (see also Fig. 3):

$$\emptyset \xrightarrow{b} q \qquad q \xrightarrow{r\alpha} m \qquad q \xrightarrow{r(1-\alpha)} \mu \ , \tag{21}$$

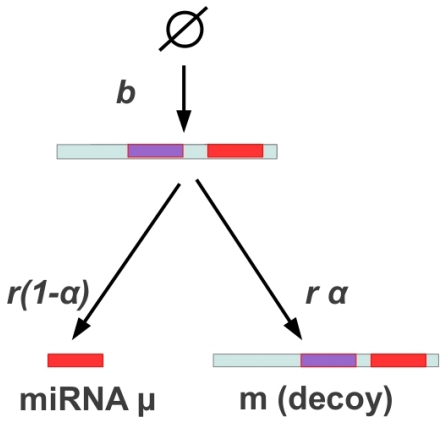

Figure 3: Schematic representation of the model of a miRNA-decoy transcript.

including transcription of the long non-coding RNA q at rate b, transport of q to the cytoplasm with processing into mature miRNA μ at rate $(1-\alpha)r$, and transport of q to the cytoplasm αr. The quantity $1-\alpha \in [0,1]$ thus gives the fraction of miRNA produced over the total number of transcribed RNAs.

At stationarity, the miRNA and the lncRNA m are produced at constant rates according to

$$\dot{m} = b\alpha \tag{22}$$

$$\dot{\mu} = b(1-\alpha) \tag{23}$$

If noise affects both the transcription rate b and the processing efficiency α (taking again Gaussian distributions with means $\overline{b}$ and $\overline{\alpha}$ and variances σ_b^2 and σ_α^2, respectively), the covariance between production rates is easily seen to be given by

$$\overline{\dot{m}\dot{\mu}} - \overline{\dot{m}}\,\overline{\dot{\mu}} = \sigma_b^2(\overline{\alpha} - \overline{\alpha^2}) - \sigma_\alpha^2\overline{b^2} \tag{24}$$

Hence noise in b and α induces noise at the level of molecular concentrations, yielding either positive or negative correlations between the steady state production rates of the miRNA μ and of the decoy m as shown in Figure 4. (Clearly, this conclusion holds as long as the noise on α is sufficiently small, or A is not too close to 1.)

These correlations, in turn, can result in a change of steady state fluctuations of other competing RNAs through the usual miRNA-mediated channels. In the case of muscle differentiation discussed in [3], large levels of noise at the transcriptional or at the processing level could be exploited in order to increase cell variability and

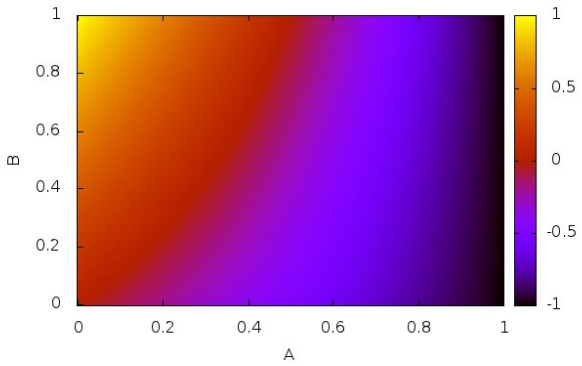

Figure 4: Pearson correlation coefficient between the production rate of miRNA μ and of decoy m, for different values of the processing noise level ($A \equiv \sigma_\alpha^2/[\alpha(1-\alpha)]$ on the x axis) and of the transcription noise level ($B \equiv \sigma_b^2/b^2$ on the y axis). High level of processing noise gives rise to negative correlations, while low level of processing noise and high level of transcriptional noise result in positive correlations.

give rise to the differentiation program. Such a mechanism could be shared by other miRNA genes representing a widespread network motif.

On the significance of the conditional mutual information as a means to signal cross-talk

Consider a system (t, m, μ) of 2 ceRNAs (a target t and a modulator m and N background targets) and one miRNA μ, subject to transcriptional fluctuations. Let us say that the experimental readouts concern the quantities

$$[m]_{xp} = [m] + [c_m] \tag{25}$$

$$[t]_{xp} = [t] + [c_t] \tag{26}$$

$$[\mu]_{xp} = [\mu] + [c_m] + [c_t] \tag{27}$$

where $[c_t]$ and $[c_m]$ represent the levels of miRNA-target and miRNA-modulator complexes, respectively. Suppose that both complexes decay catalytically, i.e. that the rates of stoichiometric complex degradation $\sigma_m = \sigma_t = 0$. In such conditions

no cross-talk is possible at steady state. Furthermore, let us assume that the transcription rates b_t, b_m, and β are drawn from a probability distribution $P_0(b_t, b_m, \beta)$ such that

$$P_0(b_t, b_m, \beta) \equiv P(b_t, \beta)\delta(b_m - k) \tag{28}$$

with P an unspecified probability distribution with finite covariance (i.e., that the target and miRNA transcription rates are random variables while the modulator transcription rate is fixed at k). We want to show that, in this case, $\Delta I([t]_{xp}, [\mu]_{xp}; [m]_{xp}) > 0$ (with ΔI defined in (37) of the Main Text) necessarily. This would imply that the condition $\Delta I([t]_{xp}, [\mu]_{xp}; [m]_{xp}) > 0$ cannot be considered as a sufficient condition for cross-talk, since knowledge of $[m]_{xp}$ can increase the mutual dependence between $[\mu]_{xp}$ and $[t]_{xp}$ even in absence of cross-talk.

To see this, note that the measured steady state levels are stochastic variables which depend on the transcription rates as

$$[m]_{xp} = f_m(\beta) \tag{29}$$

$$[\mu]_{xp} = f_\mu(b_t, \beta) \tag{30}$$

$$[t]_{xp} = f_t(b_t, \beta) \tag{31}$$

(with f_m, f_μ and f_t unspecified functions). Now let us focus on (29) and (30). Given their monotonicity with respect to each of the variables on which they depend, they can be inverted:

$$\beta = f_m^{-1}([m]_{xp}) \tag{32}$$

$$b_t = f_\mu^{-1}([\mu]_{xp}, \beta) \tag{33}$$

Hence it is possible to express $[t]_{xp}$ as a function of $[m]_{xp}$ and $[\mu]_{xp}$ directly: $[t]_{xp} = h([m]_{xp}, [\mu]_{xp})$. In other terms, one finds a deterministic dependence of $[t]_{xp}$ on $[m]_{xp}$. This implies that for each fixed $[m]_{xp}$ the mutual information between $[t]_{xp}$ and $[\mu]_{xp}$ diverges. As a consequence, their mutual information averaged over $[m]_{xp}$, $\left\langle I([t]_{xp}, [\mu]_{xp}) \right\rangle_{[m]_{xp}}$, diverges as well. At the same time, however, the mutual information between $[t]_{xp}$ and $[\mu]_{xp}$ stays finite due to the noise on b_t and β. Hence

$$\Delta I([t]_{xp}, [\mu]_{xp}; [m]_{xp}) \equiv \left\langle I([t]_{xp}, [\mu]_{xp}) \right\rangle_{[m]_{xp}} - I([t]_{xp}, [\mu]_{xp}) > 0 \tag{34}$$

necessarily.

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