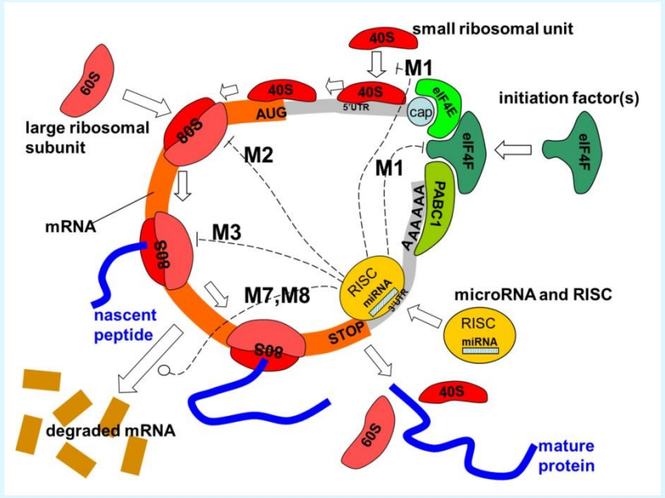
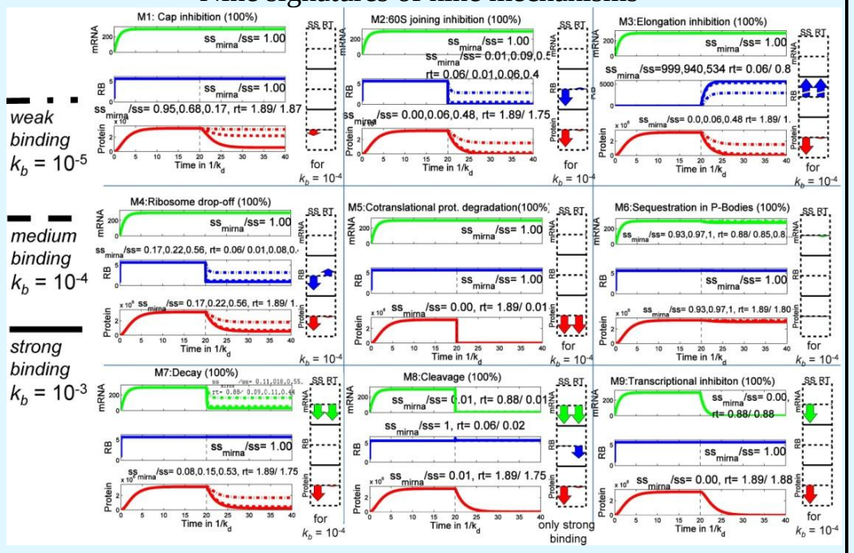
# Kinetic signatures of microRNA mechanisms (A. Zinovyev, N. Morozova, A.N. Gorban, A. Harel-Belan)

## Interaction of microRNA with protein translation process




Several mechanisms of translation repression are shown:
**M1)** on the initiation process, preventing assembling of the initiation complex or recruiting the 40S ribosomal subunit;
**M2)** on the ribosome assembly;
**M3)** on the translation process;
**M7, M8)** on the degradation of mRNA.
Here, 40S and 60S are light and heavy components of the ribosome, 80S is the assembled ribosome bound to mRNA, eIF4F is an translation initiation factor, PABC1 is the Poly-A binding protein, "cap" is the mRNA cap structure needed for mRNA circularization (which can be the normal m7G-cap or artificial modified A-cap). The initiation of mRNA can proceed in a cap-independent manner, through recruiting 40S to IRES (Internal Ribosome Entry Site) located in 5'UTR region. The actual work of RNA silencing is performed by RISC (RNA-induced silencing complex) in which the main catalytic subunit is one of the Argonaute proteins (AGO), and miRNA serves as a template for recognizing specific mRNA sequences.

## Nine signatures of nine mechanisms

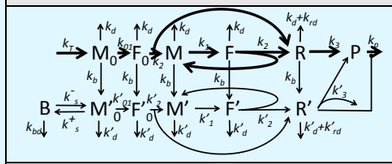
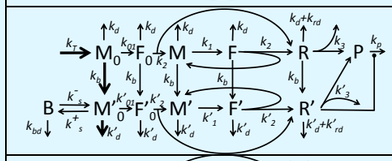
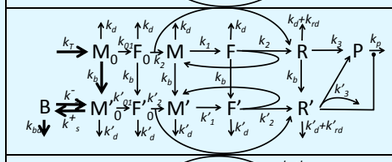
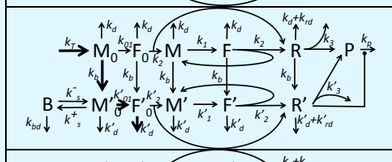
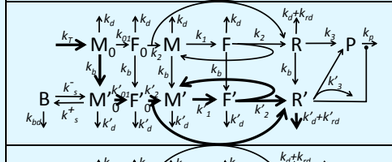
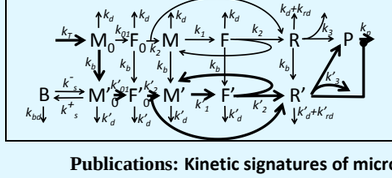



Each plot shows dynamics of three quantities: amount of mRNA (*mRNA*), average number of ribosomes per translated mRNA (*RB*), total amount of protein (*Protein*) in the time units measured in 1/$k_d$. The dynamics on the left from the dashed line shows translation without miRNA which is added at the time point 20. Three scenarios are simulated for each signature: strong, medium and weak binding strength of miRNA to mRNA. The numbers on the graphs shows relative change in the steady state ($ss_{miRNA}/ss$) and change in the relaxation time (*rt*, measured in 1/$k_d$). If three numbers are shown separated by comma, they correspond to weak, medium and strong miRNA binding. If only one number is shown, it means that the binding strength does not affect this quantity significantly. The diagrams on the right from the dynamics plot visualize values of six numbers (relative changes of steady state (SS) and relaxation time (RT) for three measurable quantities) for medium binding strength.

## Mechanisms of microRNA actions

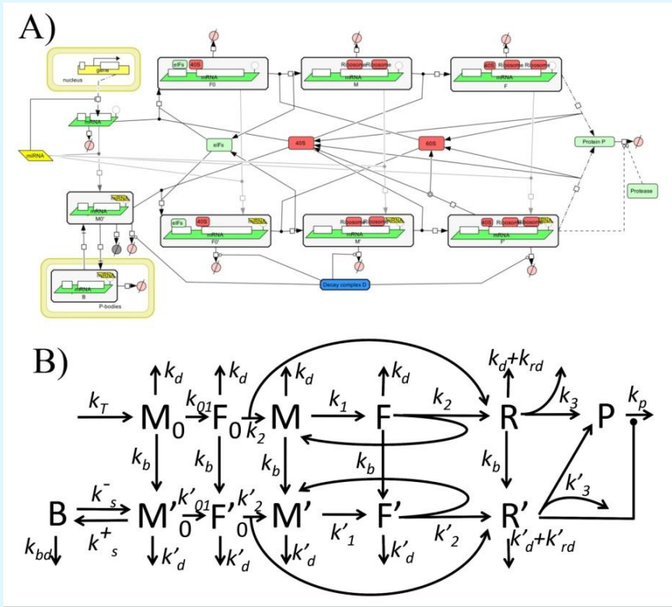
**MicroRNAs (miRNAs)** ***are key regulators of all important biological processes***, including development, differentiation and cancer. Although remarkable progress has been made in deciphering the mechanisms used by miRNAs to regulate translation, ***many contradictory findings have been published*** that stimulate active debate in this field. Here we contribute to this discussion in three ways.
**First,** based on a comprehensive analysis of the existing data, we develop a model in which all proposed mechanisms of microRNA action may coexist. Among several co-existing miRNA mechanisms, the one that will effectively be measurable is that which acts on a ***sensitive parameter of the translation process.***
**Second,** we have created a mathematical model which combines nine known mechanisms of miRNA action and estimated the model parameters from the known data.
**Third**, based on the mathematical modelling, ***we have found sensitive parameters*** of the translation process for various conditions and developed a tool for discriminating among different possible individual mechanisms of miRNA action based on translation kinetics data that can be experimentally measured (***kinetic signatures***).
**Mechanisms M1-M9:**
M1: Cap-40S Initiation Inhibition
M2: 60S Ribosomal Unit Joining Inhibition
M3: Elongation Inhibition
M4: Ribosome Drop-off (premature termination)
M5: Co-translational Nascent Protein Degradation
M6: Sequestration in P-bodies
M7: mRNA Decay (destabilisation)
M8: mRNA Cleavage
M9: Transcriptional Inhibition through microRNA-mediated chromatin reorganization following by gene silencing

## Dominant paths of the unified model of microRNA mechanisms

| Dominant path | Biological interpretation | Corresponding miRNA-mediated translation repression mechanism(s) |
|---|---|---|
|  | **$M_0F_0MFRP$**<br>normal translation with negligible effect of miRNA | None |
|  | **$M_0M'_0$**<br>the dominant effect is degradation of mRNA by miRNA | M1: Cap inhibition<br>M7: Decay<br>M8: Cleavage |
|  | **$M_0M'_0B$**<br>mRNA is captured in P-bodies | M6: Sequestration of mRNA in P-Bodies |
|  | **$M_0M'_0F'_0$**<br>mRNA translation is stuck after initiation, before the assembly of the ribosome | M2: 60S subunit joining inhibition |
|  | **$M_0M'_0F'_0M'F'R'$**<br>mRNA is stuck with ribosomes on it and destroyed, or mRNA translation is prematurely aborted | M3: Elongation inhibition<br>M4: Ribosome drop-off |
|  | **$M_0M'_0F'_0M'F'R'P$**<br>protein synthesis in the presence of miRNA with low mRNA degradation | M1: Cap inhibition<br>M2: 60S subunit joining inhibition<br>M3: Elongation inhibition<br>M5: Co-translational protein degradation mechanisms |


**Publications: Kinetic signatures of microRNA modes of action,** RNA, **Vol. 18, No. 9 (2012).**
**(arXiv:1202.1243 [q-bio.MN])**
**BMC Systems Biology, 2010 4:13. ( arXiv:0911.1797 [q-bio.MN])**


## Kinetic model with all nine mechanisms of miRNA




A) Graphical presentation of the model in the SBGN standard;
B) Schematic model presentation in the assumption that ribosomal subunits and initiation factors are present in excess.
[M0] – new synthesized and not yet initiated mRNA
[F0] – new initiated mRNA, with initiation complex, including 40S ribosomal subunit
[M] – initiated mRNA with free translation initiation site
[F] – initiated mRNA with translation initiation site occupied by 40S ribosomal subunit
[R] – number of ribosomes fully assembled on miRNA-free mRNA
[M'0] – new synthesized not initiated mRNA with one or more miRNAs bound
[F'0] – new mRNA with initiation complex, including 40S ribosomal subunit, with miRNA(s) bound to mRNA
[M'] – initiated miRNA-bound mRNA with free translation initiation site
[F'] – initiated miRNA-bound mRNA with translation initiation site occupied by 40S ribosomal subunit
[R'] – ribosomes fully assembled on miRNA-bound mRNA
[P] – protein, completely translated from the given mRNA
[B] – mRNA sequestered in P bodies.

$$
\begin{cases}
\frac{d[M_0]}{dt} = k_t - (k_d + k_{01} + k_b)[M_0] \\
\frac{d[F_0]}{dt} = k_{01}[M_0] - (k_d + k_2 + k_b)[F_0] \\
\frac{d[M]}{dt} = k_2([F_0] + [F]) - (k_d + k_1 + k_b)[M] \\
\frac{d[F]}{dt} = k_1[M] - (k_d + k_2 + k_b)[F] \\
\frac{d[R]}{dt} = k_2([F_0] + [F]) - (k_d + k_{rd} + k_3 + k_b)[R] \\
\frac{d[M'_0]}{dt} = k_b[M_0] - (k'_d + k'_{01})[M'_0] - (k_{+s}[M] - k_{-s}[B]) \\
\frac{d[F'_0]}{dt} = k_b[F_0] + k'_{01}[M'_0] - (k'_d + k'_2)[F'_0] \\
\frac{d[M']}{dt} = k_b[M] + k'_2([F'_0] + [F']) - (k'_d + k'_1)[M'] \\
\frac{d[F']}{dt} = k_b[F] + k'_1[M] - (k'_d + k'_2)[F] \\
\frac{d[R']}{dt} = k_b[R] + k'_2([F_0] + [F]) - (k'_d + k'_{rd} + k'_3)[R] \\
\frac{d[P]}{dt} = k_3[R] + k'_3[R'] - (k_p + k_r[R'])[P] \\
\frac{d[B]}{dt} = k_{+s}[M'] - k_{-s}[B] - k_{bd}[B]
\end{cases}
$$