

Systemic impact of microRNAs in animals

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microRNA target identification

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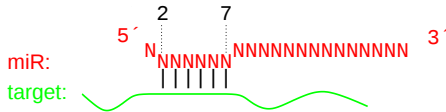
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microRNA target identification



→ the "seed"

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Identification of miRNA targets

Computational programs for target prediction: look for seed matches in 3' UTRs, select the ones that were conserved in evolution.

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Identification of miRNA targets

Computational programs for target prediction: look for seed matches in 3' UTRs, select the ones that were conserved in evolution.

Such short matches are very frequent (60 % of human coding genes seem to be targeted: Friedman *et al.*, 2009).

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⇒ miRNAs are implicated in every physiological process in animals.

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Identification of miRNA targets

Computational programs for target prediction: look for seed matches in 3' UTRs, select the ones that were conserved in evolution.

Such short matches are very frequent (60 % of human coding genes seem to be targeted: Friedman *et al.*, 2009).

⇒ miRNAs are implicated in every physiological process in animals.

miRNA-mediated repression is very modest (usually < 2-fold): have these binding sites been conserved because they trigger a butterfly effect (large consequences for minor causes)?

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An alternative hypothesis

- ▶ Most computationally predicted targets are not *functionally* targeted (not repressed enough).

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An alternative hypothesis

- ▶ Most computationally predicted targets are not *functionally* targeted (not repressed enough).
- ▶ Their phylogenetic conservation means that these binding sites have a function.

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An alternative hypothesis

- ▶ Most computationally predicted targets are not *functionally* targeted (not repressed enough).
- ▶ Their phylogenetic conservation means that these binding sites have a function.
- ▶ That function could be to repress the miRNA by titrating it.

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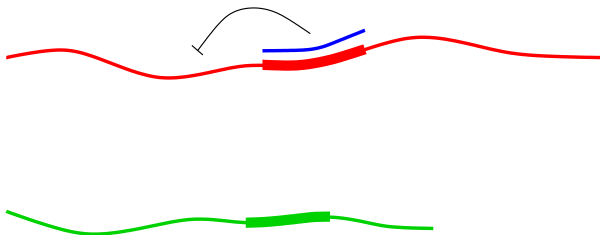
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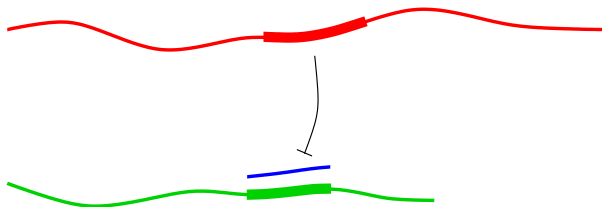
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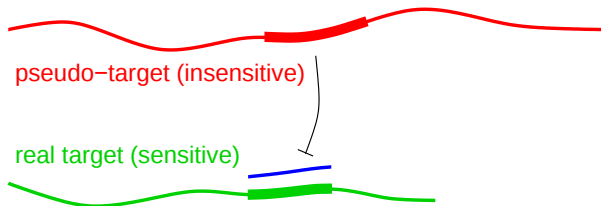
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A new interpretation

The molecular event is the same (interaction between an mRNA and a miRNA), but the interpretation of that interaction is different:

- ▶ Current theory: the miRNA represses the mRNA.
- ▶ New hypothesis: the mRNA represses the miRNA (except for a few real targets).

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Discriminative predictions

According to the new hypothesis:

According to the current theory:

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Discriminative predictions

According to the new hypothesis:

- ▶ Abundantly expressed pseudo-targets have a stronger miRNA-modulating effect, so their interaction with the miRNA should be better conserved

According to the current theory:

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Discriminative predictions

According to the new hypothesis:

- ▶ Abundantly expressed pseudo-targets have a stronger miRNA-modulating effect, so their interaction with the miRNA should be better conserved $\implies$ mRNA abundance should be positively correlated with miRNA binding site conservation.

According to the current theory:

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Discriminative predictions

According to the new hypothesis:

- ▶ Abundantly expressed pseudo-targets have a stronger miRNA-modulating effect, so their interaction with the miRNA should be better conserved $\implies$ mRNA abundance should be positively correlated with miRNA binding site conservation.

According to the current theory:

- ▶ Each target is a real target, its interaction is more or less conserved depending on the functional importance of the regulation (it is not expected to correlate with gene expression).

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mRNA abundance and seed match conservation

Does mRNA abundance correlate positively with miRNA binding site conservation?

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mRNA abundance and seed match conservation

Does mRNA abundance correlate positively with miRNA binding site conservation?

miRNA binding site conservation: measured by TargetScan's " P_{CT} " score. mRNA abundance in published microarray experiments.

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mRNA abundance and seed match conservation

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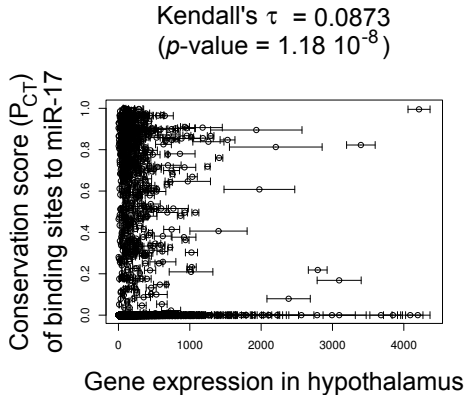
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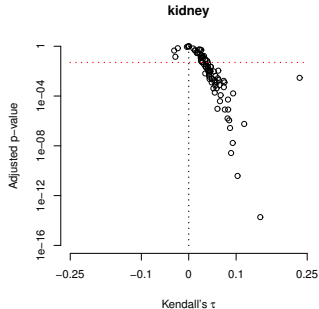
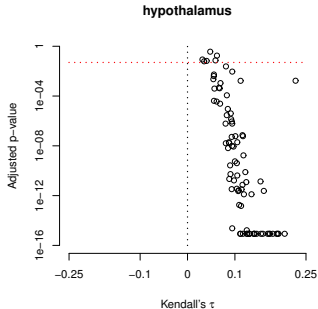
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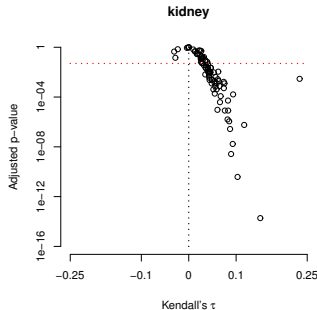
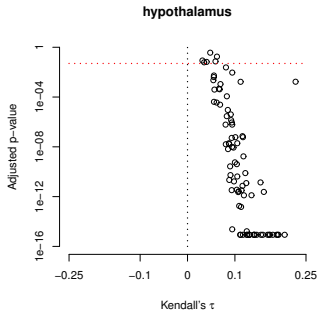
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+34 other mouse tissues, same results:

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+33 fly tissues, same results:

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Discriminative predictions

According to the new hypothesis:

According to the current theory:

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Discriminative predictions

According to the new hypothesis:

- ▶ Pseudo-target expression levels can fluctuate between individuals in a natural population (phenotype is robust).

According to the current theory:

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Discriminative predictions

According to the new hypothesis:

- ▶ Pseudo-target expression levels can fluctuate between individuals in a natural population (phenotype is robust).

According to the current theory:

- ▶ miRNA targets are tightly regulated (inter-individual fluctuation should not exceed miRNA-guided repression).

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Natural variability vs. miRNA-guided repression

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Natural variability vs. miRNA-guided repression

Baek *et al.*, 2008: quantification of miR-223-mediated repression in mouse neutrophils.

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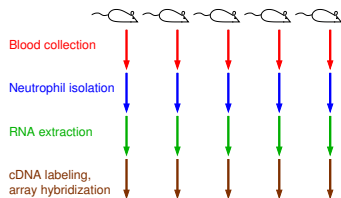
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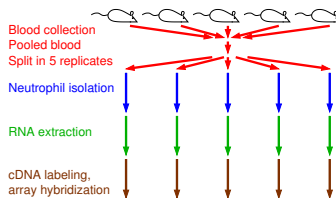
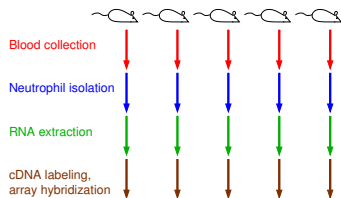
Natural variability vs. miRNA-guided repression

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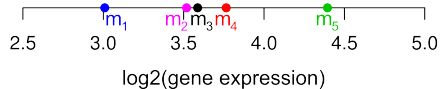
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Baek *et al.*, 2008: quantification of miR-223-mediated repression in mouse neutrophils.



Natural variability vs. miRNA-guided repression

Gene *Styx*



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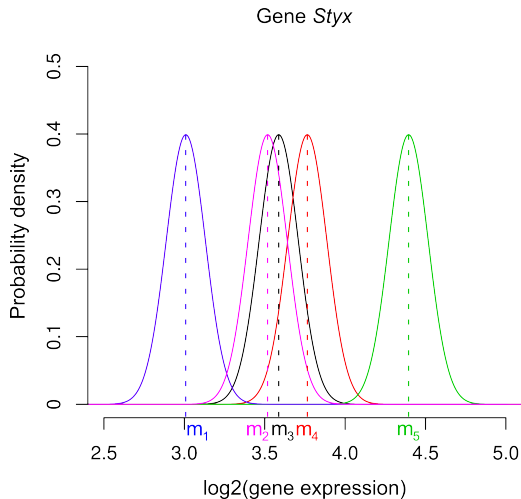
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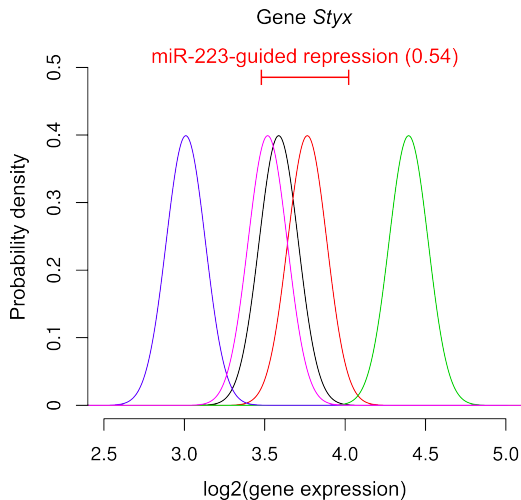
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Natural variability vs. miRNA-guided repression

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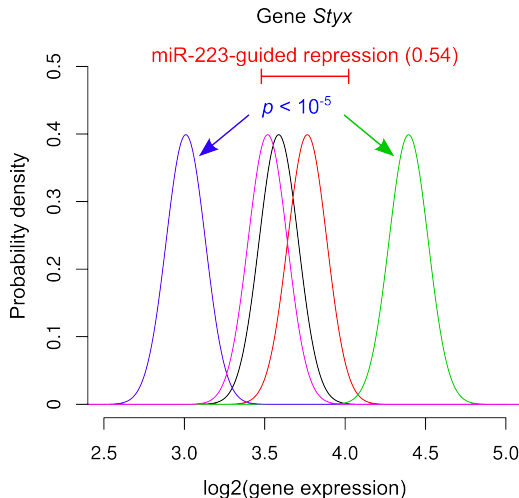
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Natural variability vs. miRNA-guided repression



p : probability that the difference between two individual mice is smaller than miRNA-guided repression

Natural variability vs. miRNA-guided repression

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Natural variability vs. miRNA-guided repression

For 168 predicted targets out of 189:
inter-individual fluctuations across 5 wild-type mice exceeds
miRNA-mediated regulation (p -value < 0.05).

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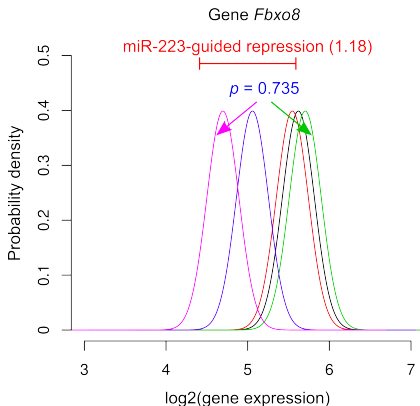
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Natural variability vs. miRNA-guided repression

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Natural variability vs. miRNA-guided repression

Gene	Quantified miRNA effect (Baek et al., 2008)	<i>p</i> -value
Arfp1	1.270	0.340
Arpp19	0.873	0.0675, 0.246, 0.426
Fam46a	1.500	0.0732
Fbxo25	1.530	0.0667
Fbxo8	2.270	0.735
Frmd4a	1.28	0.0937
Lelp1	0.889	0.078
Mospd1	1.22	0.0743
Nlrp3	1.24	0.432
Pik3c2a	0.541	0.682, 0.747, 0.969
Plce1	0.911	0.256
Rc3h1	1.07	0.0732
...	...	...

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Generalization

The pseudo-target theory can be generalized to every regulator (transcription factors, RNA-binding proteins,...).

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The pseudo-target theory can be generalized to every regulator (transcription factors, RNA-binding proteins,...). Molecular biology and comparative genomics can identify intermolecular interactions, but they don't tell whether the function is to regulate the target, or to titrate the regulator.

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Transcription factor Zfy-overexpressing mice (Hélène Royo, Turner lab, MRC, London). Transgene is overexpressed in the testis from day 13.5, cells enter apoptosis at day 15.5. mRNA quantification by microarray at these two stages.

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Transcription factor Zfy-overexpressing mice (Hélène Royo, Turner lab, MRC, London). Transgene is overexpressed in the testis from day 13.5, cells enter apoptosis at day 15.5. mRNA quantification by microarray at these two stages.

Zfy binds the [A/T]GGCC pentamer.

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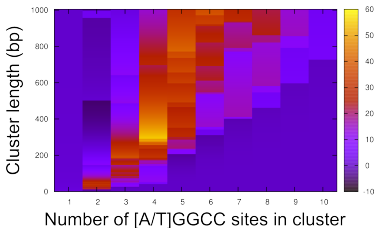
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Transcription factor Zfy-overexpressing mice (Hélène Royo, Turner lab, MRC, London). Transgene is overexpressed in the testis from day 13.5, cells enter apoptosis at day 15.5. mRNA quantification by microarray at these two stages.

(% of up-regulated genes with cluster) - (% of unaffected genes with cluster)



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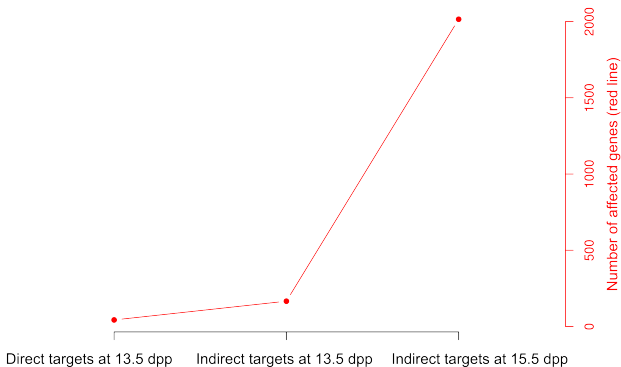
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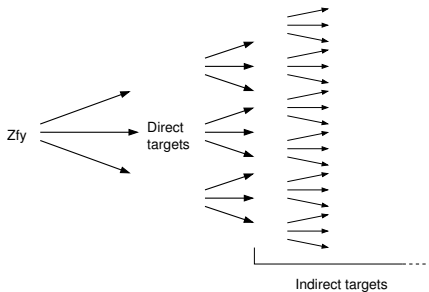
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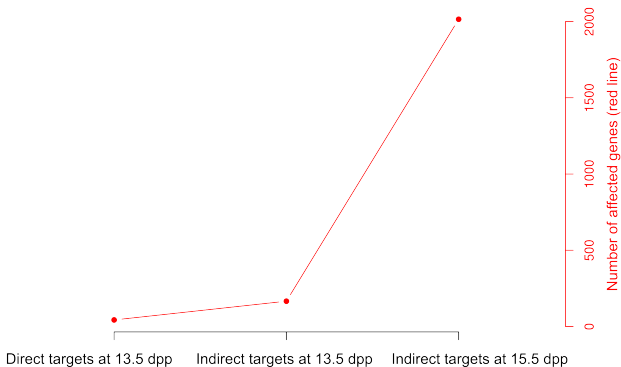
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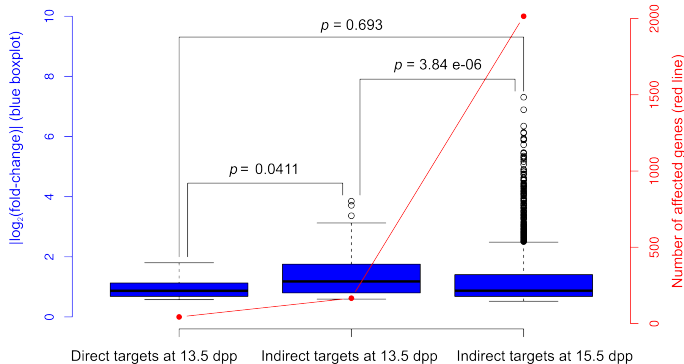
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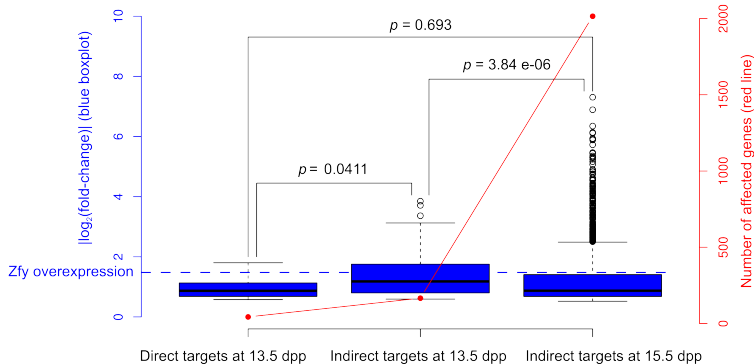
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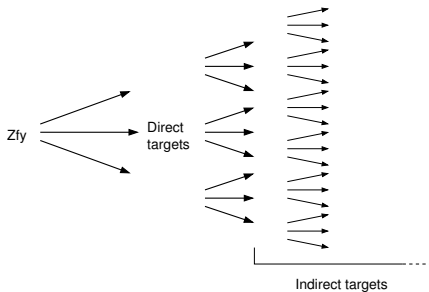
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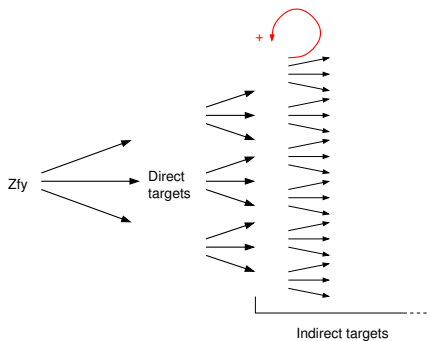
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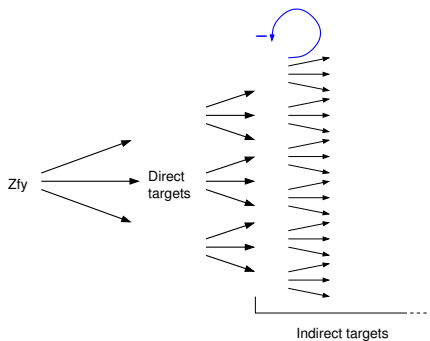
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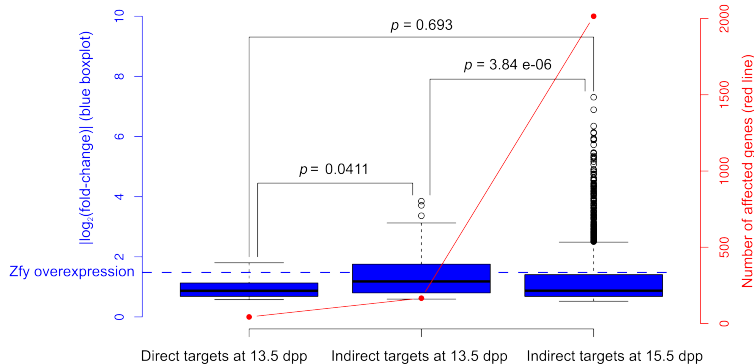
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Conclusion: revisiting miRNA target definition

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Conclusion: revisiting miRNA target definition

Every measurable change in gene expression does not translate into a macroscopic, evolutionarily selectable phenotype.

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Conclusion: revisiting miRNA target definition

Every measurable change in gene expression does not translate into a macroscopic, evolutionarily selectable phenotype.

Cells can modulate miRNA activity in a cell type-specific manner (integrating expression information from hundreds of genes on a single regulator)

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Conclusion: revisiting “gene regulation” definition

High-throughput identification of transcription factor or RNA-binding protein targets: thousands of genes are bound, many of them are not under selective pressure to keep these binding sites.

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Conclusion: revisiting “gene regulation” definition

High-throughput identification of transcription factor or RNA-binding protein targets: thousands of genes are bound, many of them are not under selective pressure to keep these binding sites.

Microscopic events which are neutral in evolutionary terms.

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Conclusion: revisiting “gene regulation” definition

High-throughput identification of transcription factor or RNA-binding protein targets: thousands of genes are bound, many of them are not under selective pressure to keep these binding sites.

Microscopic events which are neutral in evolutionary terms.

Are there pseudo-targets for these other regulators?

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Conclusion: revisiting “gene regulation” definition

High-throughput identification of transcription factor or RNA-binding protein targets: thousands of genes are bound, many of them are not under selective pressure to keep these binding sites.

Microscopic events which are neutral in evolutionary terms.

Are there pseudo-targets for these other regulators?

A possible methodology: quantification of gene expression changes after perturbation of a regulator (attenuation and amplification along the affected pathways).

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High-throughput identification of transcription factor or RNA-binding protein targets: thousands of genes are bound, many of them are not under selective pressure to keep these binding sites.

Microscopic events which are neutral in evolutionary terms.

Are there pseudo-targets for these other regulators?

A possible methodology: quantification of gene expression changes after perturbation of a regulator (attenuation and amplification along the affected pathways).

A central feature of biological systems: their robustness to external insults. Hard to reconcile with the extreme sensitivity required for fine-tuning (the “butterfly effect” has probably been counter-selected).

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Anna Sergeeva



Laura Martinez



Natalia
Pinzón Restrepo



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► Robustness of biological pathways

► Conservative approximations in the assessment of abundance/conservation correlation

► Positive correlation between gene expression and conservation of miRNA binding sites

► Identification of Zfy binding sites

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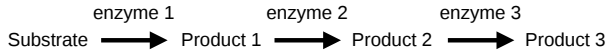
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Second paradox



mRNA abundance and seed match conservation

- ▶ Most predicted targets are expected to be pseudo-targets (conservative approximation: we will consider every predicted target).
- ▶ miRNA binding site should correlate with that mRNA's abundance in the cells where miRNA titration is beneficial (conservative approximation: we will consider whole tissues and organs).
- ▶ Poorly abundant mRNAs (without a real titration effect on the miRNA) should not exhibit such correlation (conservative approximation: we will consider every mRNA).

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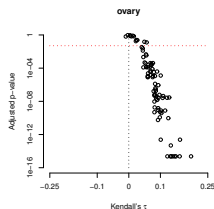
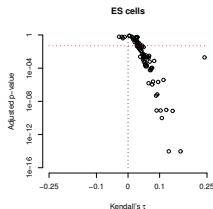
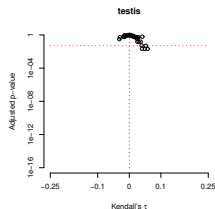
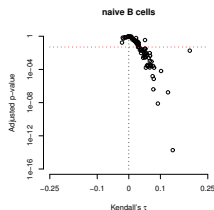
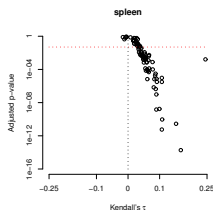
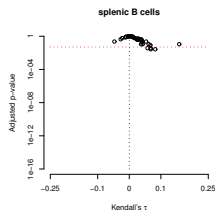
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mRNA abundance and seed match conservation

Systemic impact of
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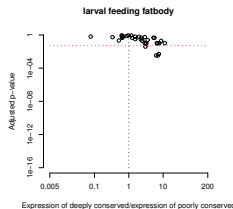
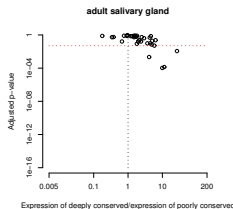
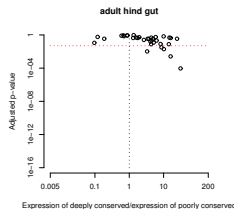
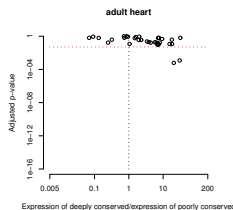
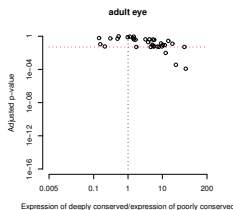
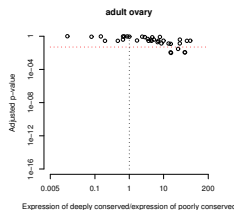
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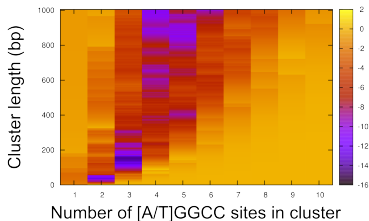
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Generalization

(% of down-regulated genes with cluster) - (% of unaffected genes with cluster)



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