RTH
CENTER FOR NON-CODING RNA IN TECHNOLOGY AND HEALTH

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miRNA and transcription factor networks

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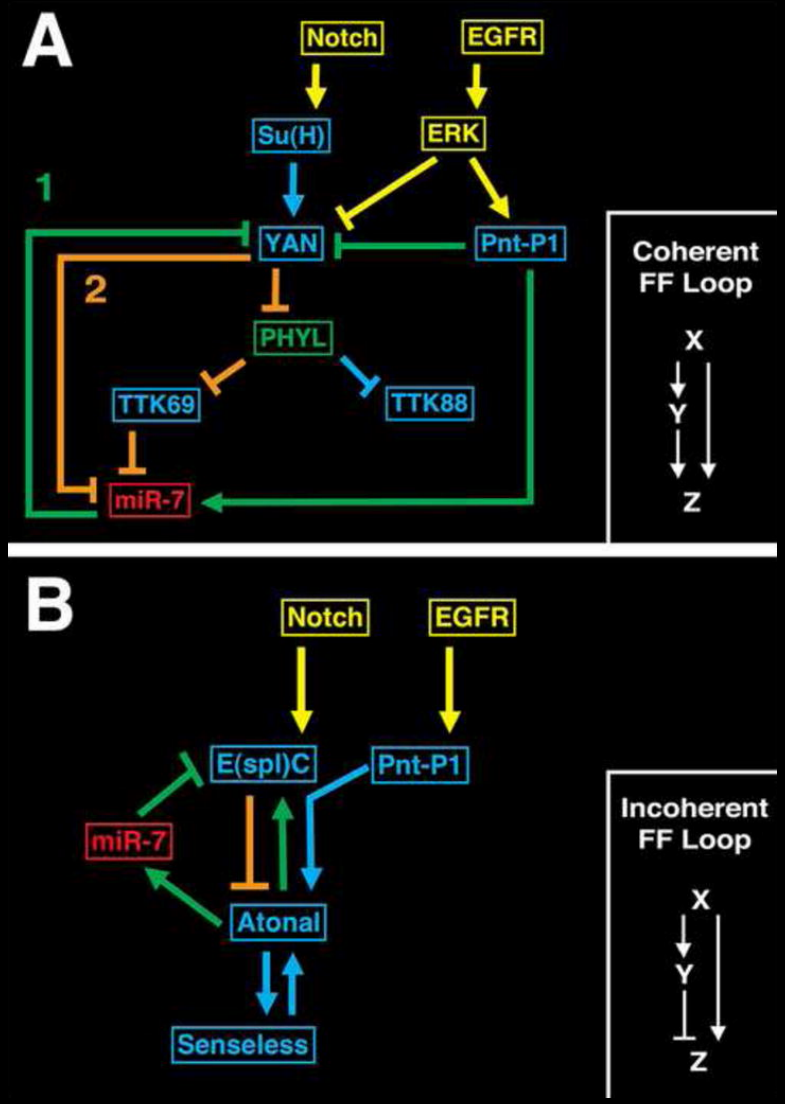
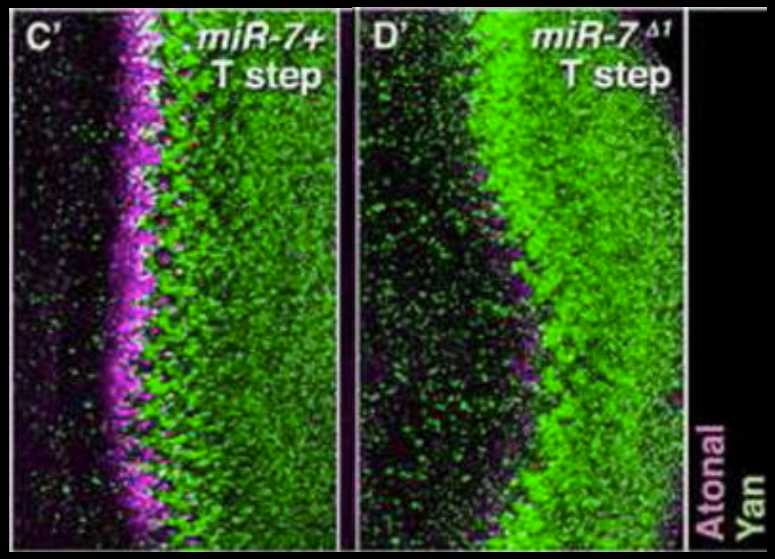
miRNA stabilization of gene regulatory networks



miRNA knockouts often don't cause differences in phenotype

Li et al. (Cell 2009) showed a mir-7 knockout created a phenotypic change only when the regulatory network is perturbed



What does the miRNA regulatory network look like?

How is it connected to the transcription factor network?

Is there evidence in the network structure that miRNAs confer robustness to the genetic regulatory network?

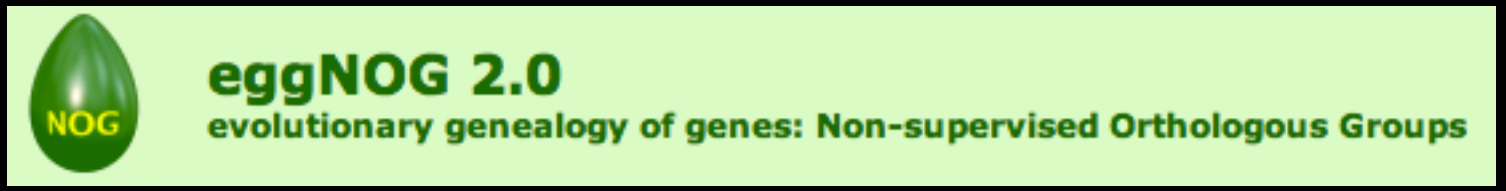
To partially answer these questions:
miRNA:mRNA interactions were collected from String and from miRNA target prediction software Pita, Pictar, MicroT, TargetScan, Miranda and RNA22

Transcription binding sites were predicted from JASPAR

A database of known and predicted protein interactions derived from four sources:

- Genomic Context
- High-throughput Experiments
- (Conserved) Coexpression
- Previous Knowledge such as textmined PubMed abstracts

We added a new miRNA dataset to String using the PubMed textmining pipeline. This produced 1800 human miRNA:protein pairs. 300 were validated manually with correct recognition in 80% of pairs.



A database of orthologous groups of genes. The orthologous groups are annotated with functional categories parsed from COG/KOG categories.

hsa-miR-7

ENSG00000149269

Cancer Res. 2008 Oct 15;68(20):8195-200.

MicroRNA-7, a homeobox D10 target, inhibits p21-activated kinase 1 and regulates its functions.

Reddy SD, Ohshiro K, Rayala SK, Kumar R.

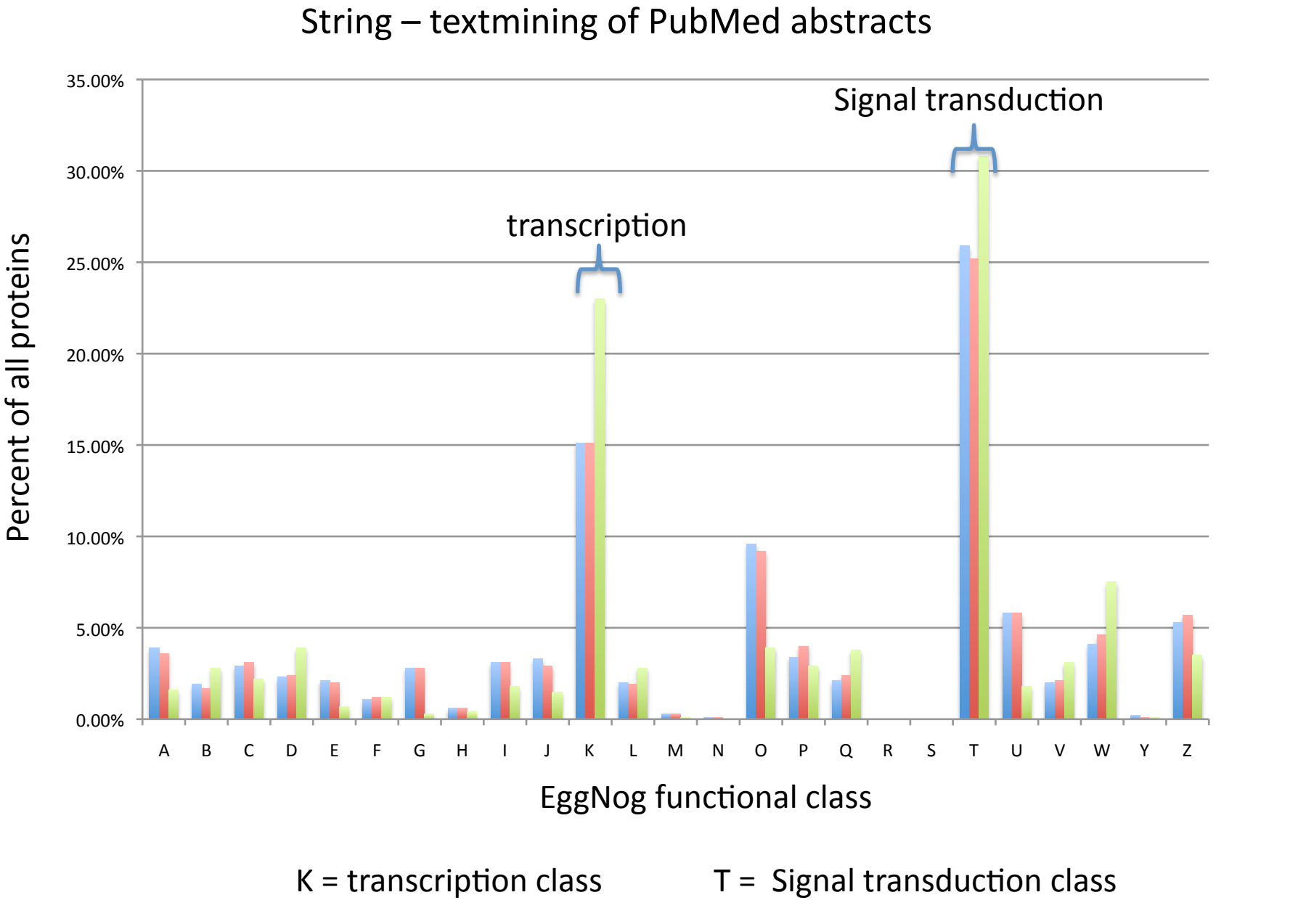
Department of Molecular and Cellular Oncology, The University of Texas MD Anderson Cancer Center, Baylor College of Medicine, Houston, Texas 77030, USA.

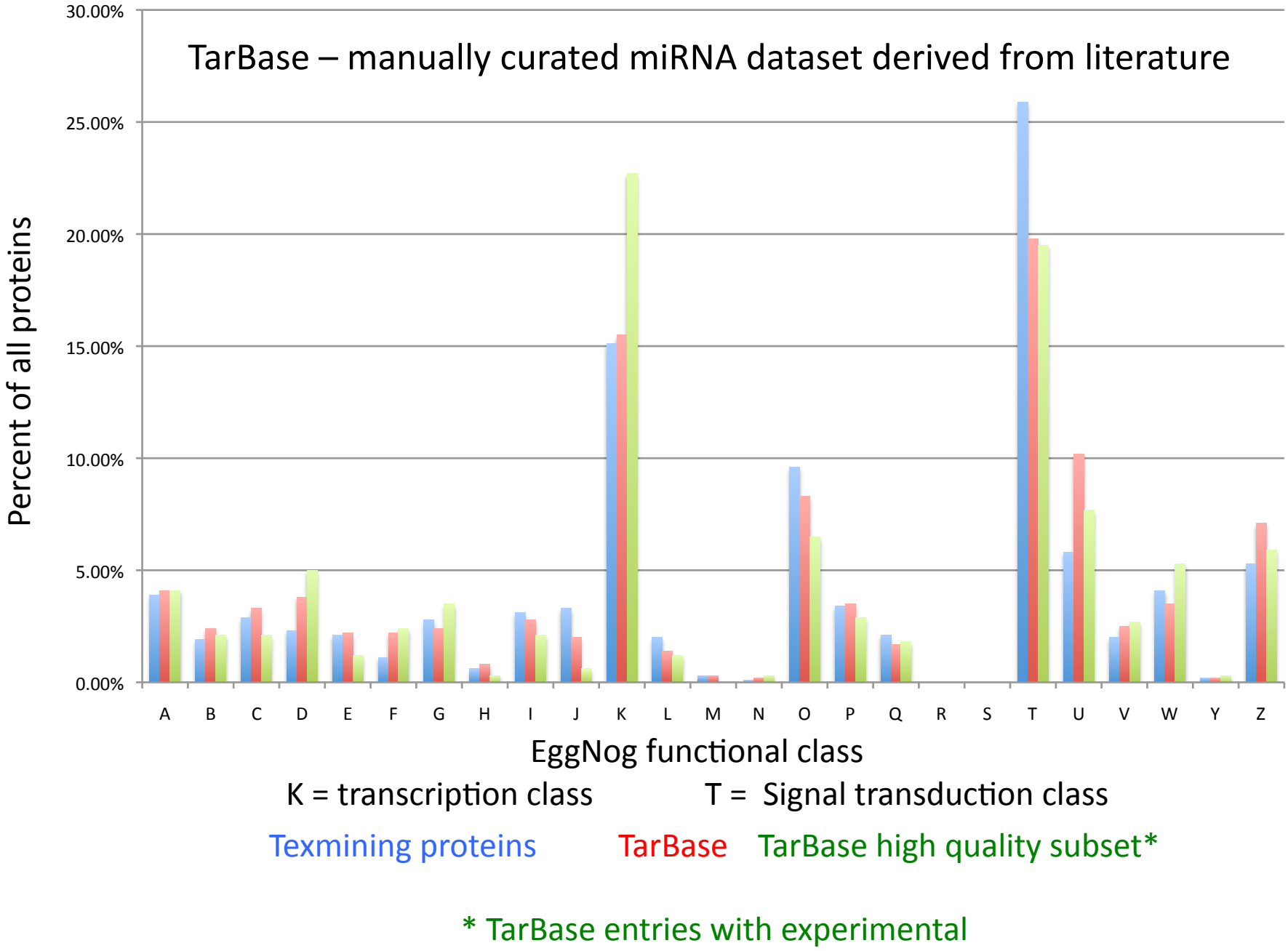
Abstract

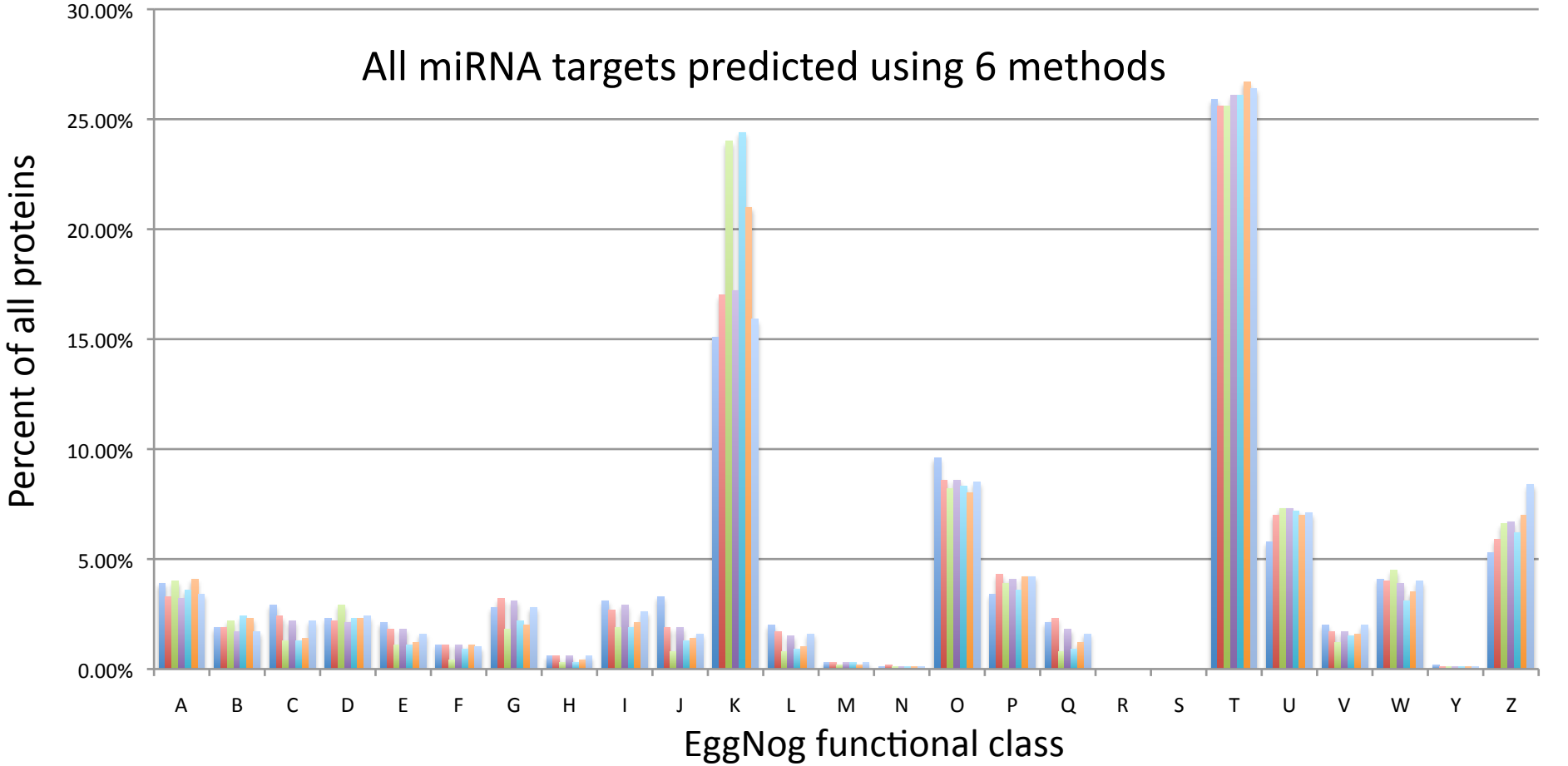
MicroRNAs are noncoding RNAs that inhibit the expression of their targets in a sequence-specific manner and play crucial roles during oncogenesis. Here we show that microRNA-7 (miR-7) inhibits p21-activated kinase 1 (Pak1) expression, a widely up-regulated signaling kinase in multiple human cancers, by targeting the 3'-untranslated region (UTR) of Pak1 mRNA. We noticed an inverse correlation between the levels of endogenous miR-7 and Pak1 expression in human cancer cells. We discovered that endogenous miR-7 expression is positively regulated by a homeodomain transcription factor, HoxD10, the loss of which leads to an increased invasiveness. HoxD10 directly interacts with the miR-7 chromatin. Accordingly, the levels of Pak1 protein are progressively up-regulated whereas those of miR-7 and its upstream activator HoxD10 are progressively down-regulated in a cellular model of breast cancer progression from low to highly invasive phenotypes. Furthermore, HoxD10 expression in highly invasive breast cancer cells resulted in an increased miR-7 expression but reduced Pak1 3'-UTR-luciferase activity and reduced Pak1 protein. Finally, we show that miR-7 introduction inhibits the motility, invasiveness, anchorage-independent growth, and tumorigenic potential of highly invasive breast cancer cells. Collectively, these findings establish for the first time that Pak1 is a target of miR-7 and that HoxD10 plays a regulatory role in modifying the expression of miR-7 and, consequently, the functions of the miR-7-Pak1 pathway in human cancer cells.

PMID: 18922890 [PubMed - indexed for MEDLINE] Free Article

PMID: 18922890


String – textmining of PubMed abstracts
35.00%
30.00%
25.00%
20.00%
15.00%
10.00%
5.00%
0.00%
Percent of all proteins
transcription
Signal transduction
A B C D E F G H I J K L M N O P Q R S T U V W Y Z
EggNog functional class
K = transcription class
T = Signal transduction class
all proteins
proteins identified by textmining
proteins with miRNA association (in PubMed abstracts)


TarBase – manually curated miRNA dataset derived from literature
Percent of all proteins
30.00%
25.00%
20.00%
15.00%
10.00%
5.00%
0.00%
A B C D E F G H I J K L M N O P Q R S T U V W Y Z
EggNog functional class
K = transcription class
T = Signal transduction class
Texmining proteins
TarBase
TarBase high quality subset*
* TarBase entries with experimental



All textmined proteins Pita Pictar MicroT TargetScan Miranda RNA22

K –transcription class T – Signal transduction class

miRNA target network summary

Textmining was used to identify miRNA suppression of mRNAs using all PubMed abstracts

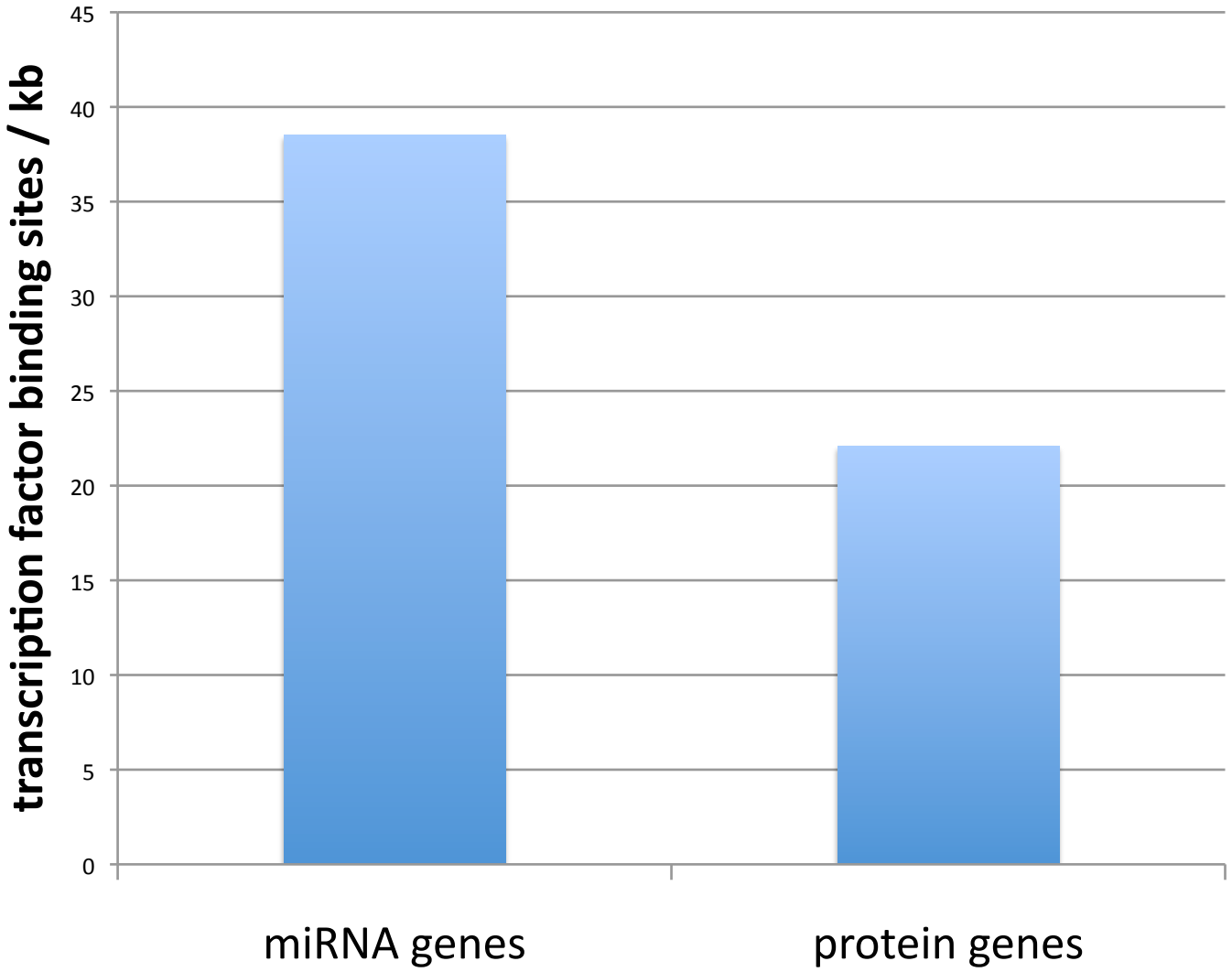
Transcription factors are preferentially targeted by miRNAs

Random textmining sets were checked by eye to validate this targeting preference

6 miRNA target prediction methods were compared. The 3 methods generally accepted as more accurate also show miRNAs preferentially target transcription factors

If miRNAs target TFs more often than other classes of gene, do TFs target miRNA genes more often than protein genes?

Predicted (JASPAR) transcription factor binding sites within 1kb of transcript start of gene



A miRNA:transcription factor network?

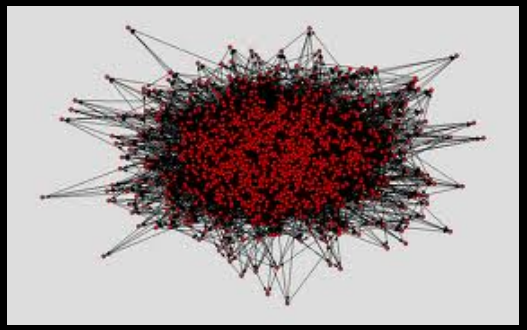
miRNAs and transcription factors target each other more than other genes

This suggests a miRNA <==> transcription factor network which we can only partially observe due to the false negative rate

The high false positive rates of TF and miRNA binding sites change the baseline but not the difference between different functional classes in the previous analyses

We partially reconstruct the human miRNA:transcription factor network based upon Pictar, TargetScan and Miranda predicted miRNA:mRNA binding and JASPAR predicted transcription factor binding sites

This produced a cyclic, directed graph with coloured edges. Each edge is “coloured” suppressive (miRNAs) or activating (transcription factor binding sites)



Human miRNA:transcription factor network analysis

Number of nodes	1,870
Number of edges	52,063
Percent activating edges	59%
Percent suppressive edges	41%

2 element loops identified by Perl hashes

Fanmod analysis of 3 element motifs

Possible role?

Type 1 coherent feedforward loop → Latch

Type 1 coherent feedforward loop → Latch

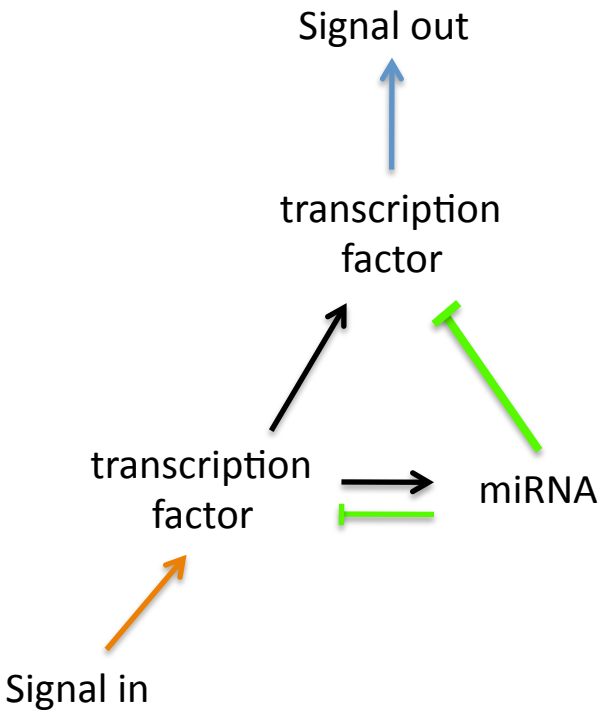
Type 1 incoherent feedforward loop with feedback → Fold change sensor with possible gain control

(Goentoro 2009)

3 element loops were identified by Fanmod using exhaustive enumeration and compared to the motif statistics of 10,000 random networks built by random edge flipping maintaining local in and out node connectivities of the original network

ID	Adj	Frequency [Original]	Mean-Freq [Random]	Standard-Dev [Random]	Z-Score	p-Value
78		0.00016176%	0.00016142%	5.8055e-10	5.8273	0
36		7.2469%	7.2317%	2.6009e-05	5.8273	0
164		0.045812%	0.045717%	1.6442e-07	5.8273	0
38		1.4862%	1.3952%	0.00016666	5.4588	0
12		25.391%	25.349%	8.2613e-05	5.0662	0
166		0.0039053%	0.0030411%	1.7265e-06	5.0056	0
6		57.199%	57.168%	8.1708e-05	3.8066	0
46		0.00069325%	0.00050931%	7.1061e-07	2.5885	0.005
102		0.006713%	0.0060415%	2.6484e-06	2.5355	0.01
102		0.0015483%	0.001286%	1.1359e-06	2.3087	0.009
38		0.19703%	0.18563%	5.536e-05	2.06	0.02

Incoherent feedforward loop with feedback



ID	Adj	Frequency [Original]	Mean-Freq [Random]	Standard-Dev [Random]	Z-Score	p-Value
46		0.00069325%	0.00050931%	7.1061e-07	2.5885	0.005

Transcriptional amplification is stochastic, showing burst patterns of "shot noise"

Noise is inherent in all physical systems (quantum, thermal, etc)

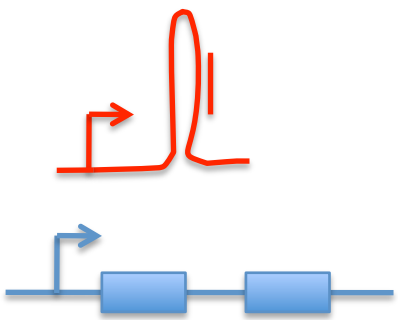
Noise, amplification and feedback are a potent mix which can easily lead to propagation and amplification of noise throughout a network at the expense of any signal

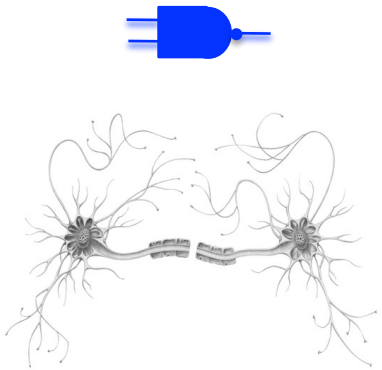
Analog computers grew to a certain size then noise propagation halted their evolution

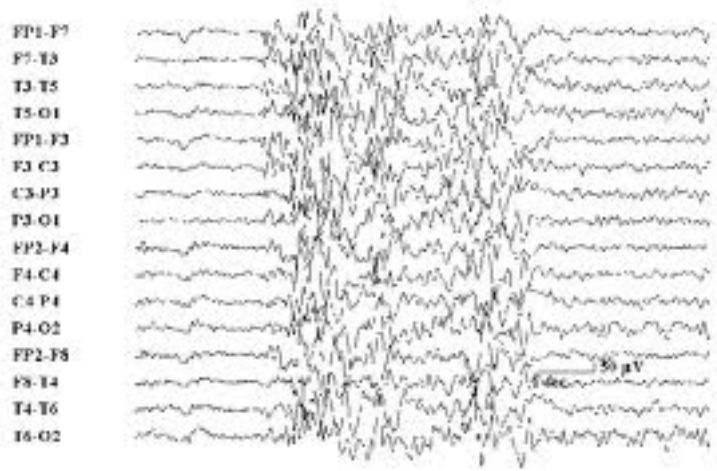
Gating is necessary in any computer to inhibit amplified feedback of noise causing a chaotic output

Genetic regulatory networks are full of feedback loops

Gating is necessary to halt noise propagation







Co-conspirators

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