Impact of microRNAs on Organization of Protein Interactions and Formation of Protein Complexes

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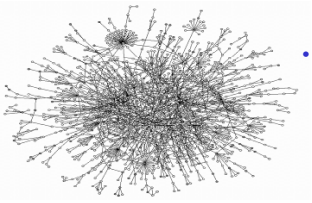
(Thanks: Wilson Goh, Guimei Liu, Judy Sng)

Motivation

- **Typical PPI network**

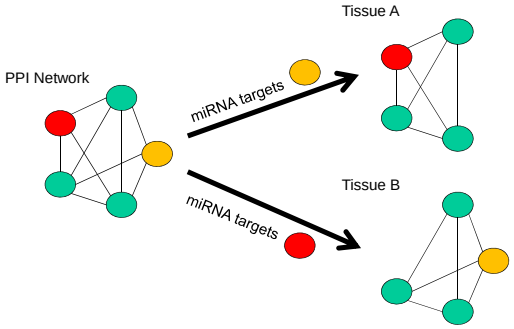


- **Can a protein interact w/ so many proteins simultaneously?**
- **Big "hub" & its "spokes" should be decomposed into subclusters**
  - A subcluster is a set proteins that interact in the same space & time
  - Viz., a protein complex

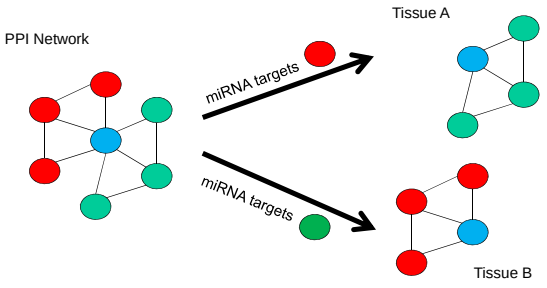
MicroRNAs

- **MiRNAs are small regulatory molecules that act by mRNA degradation or via translational repression**
- **Do miRNAs have a role in controlling complex formation?**
- **E.g., diff expression of miRNAs across diff tissues can result in formation of diff protein complexes by repressing expression of some sub-components**

Can this happen?



Can this happen?



Hypothesis

- **MiRNAs w/ widely diff expression profiles (i.e., anti-coexpressed) control mutually exclusive bio processes; and so result in diff complexes**

Results in progress…

- **Verify some general properties implied by the Hypothesis**
- **Check some predictions in experiments**

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Terminology

- **"Hub" – proteins having ≥50 PPI partners**
- **"Hub spokes" – PPI partners of a hub**
- **"Hamming distance" – # of tissues two miRNAs are differentially expressed in**
- **"Anti-coexpressed" – two miRNAs with Hamming distance ≥35 (out of max of 40 tissues)**
- **"Co-expressed" – two miRNAs that tend to be expressed in the same tissues**

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Data Sets

- **Biological network info**
  - Human Protein Reference Database
- **MiRNA expression info**
  - 40 normal tissues from Landgraf's database
- **Protein complex info**
  - CORUM
- **MiRNA target info**
  - Prediction by Diana Micro-T

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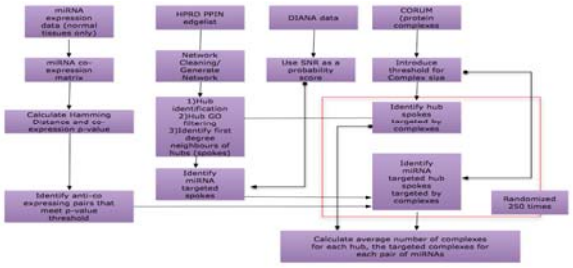
Workflow



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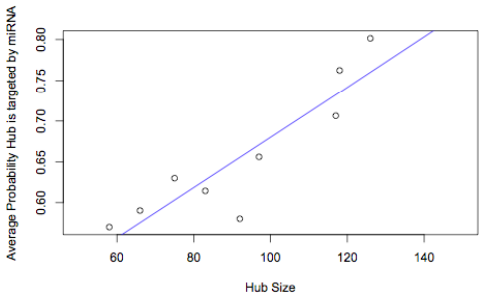
- **Size of hub (i.e., # of neighbors it has) is linearly correlated w/ ave prob of hub targeted by miRNA**



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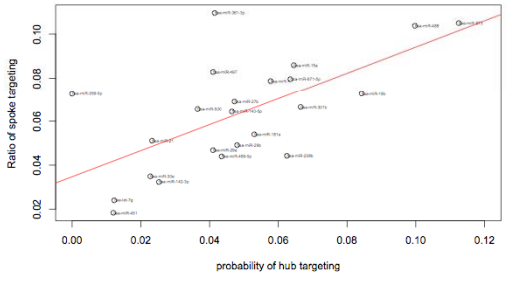
- **When a miRNA is more likely to target a hub, it is also more likely to target more of its spokes**



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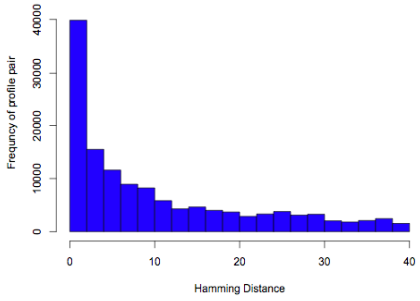
- **Freq distribution of Hamming distance based on all miRNA pairs shows that most miRNA pairs are co-expressed**



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- Co-expressed miRNAs are more freq than anti-coexpressed miRNAs
- Are hub spokes preferentially targetted by them?

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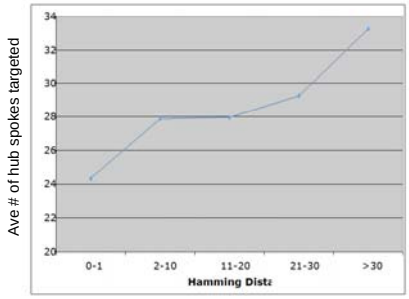
- Anti-coexpressed MiRNAs target "hub spokes" more strongly than co-expressed miRNAs



• Co-expressed miRNAs, despite much larger #, target far fewer hub-spokes than anti-coexpressed miRNAs

⇒ Anti-coexpressed miRNAs have propensity to regulate direct partners of hubs

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- Hub spokes are preferentially targeted by anti-coexpressed miRNAs
- Do they work "co-operatively" or "antagonistically"?

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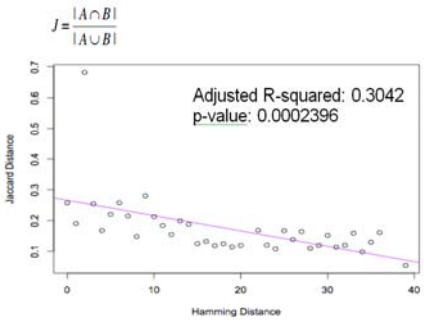
- Overlap betw targeted spokes decreases with increasing anti-coexpression



• Anti-coexpressed miRNAs avoid targeting same spokes

⇒ These miRNAs are likely to involve in opposed functions

⇒ May be tissue-specific & complexes formed resp. may be diff

⇒ Avoid targeting complex members of each other

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Spoke targeting by miRNAs is specific

- For a hub, if its spokes targeted by a miRNA (in an anti-coexpressed pair) are removed, can the remaining spokes form protein complexes?
- Yes! For every targeting miRNA (in an anti-coexpressed pair), 80-90% of the time, the remaining spokes can form protein complexes

⇒ Targetting miRNA affects small subset of protein complexes formed by hub spokes

⇒ Very specific mode of action?

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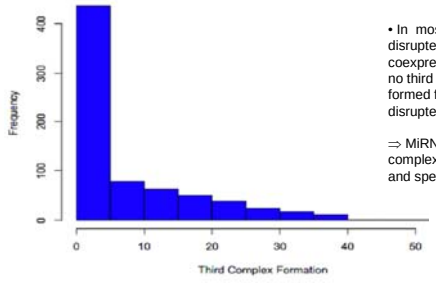
- Hub-Spoke targeting by anti-coexpressed miRNAs is specific



• In most complexes disrupted by anti-coexpression pairs, there is no third complex that can be formed from components of disrupted complexes

⇒ MiRNA disruption of complexes is a controlled and specific event

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- **Anti-coexpressed miRNAs target different subsets of complexes controlled by hubs**
- **Do these disrupted complexes have something to do with maintaining tissue-specific function or regulating tissue-specific processes?**

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Identification of miRNA-regulated complexes

- **Examine complexes disrupted in a scenario where there is evidence for miRNA upregulation and mRNA downregulation**
- **A downregulated mRNA that is predicted to be targeted by an upregulated miRNA (in an anti-coexpressed pair) is considered to be a real target**
- **Check protein complexes involving real targets**
  - Are these expected to be suppressed?

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VPA-Treated Mice

- **Valporic Acid (VPA) prompts differentiation of hippocampal neural progenitor cells into neurons, but they prevent their differentiation into oligo-dendrocytes and astrocytes**
- **Tx of mice over a 2-day period w/ VPA indicated readjustment of miRNA levels. 136 miRNAs were over-expressed with 1000 targeted genes down regulated**
- **236 genes are high-confidence miRNA targets**

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The 236 Genes

- **Genes in this set are involved in**
  - Chromatin modification (n= 14, P = 2.12e-05)
  - Nervous system dev (n = 23, P = 0.00077)
  - Cell differentiation (n = 32, P = 0.00129)

⇒ **These miRNA targets have role in epigenetic regulation in the maturation of neurons from progenitors**

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Complexes Disrupted

- **Check against CORUM shows 53 complexes got disrupted via elevated miRNA targeting**
  - 15 of these contain HDAC 1 and/or 2.
  - 8 possess Swi/Snf components involved in neuronal differentiation and neurogenesis
  - 5 belong to polycomb family of complexes which are epigenetic regulators, play a role in cell fate transition and neuronal differentiation
  - 8 complexes are SMAD regulators. SMAD can induce proliferation and differentiation of hippocampal neurons

Caveat: These are the best-matching human complexes, not mouse!

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Complexes Disrupted

HDAC-containing	Switch-involved	SMADS	Others
Anti-HDAC2	BAF/pBAF	JUND-FOSB-SMAD3-SMAD4	BRAF-RAF1-14-3-3
BRM-SIN3A-HDAC	p300-CBP-p270-SWI/SNF	PML-SMAD2/3-SARA	BRCC
BHC/BHC110	SWI-SNF_chromatin_remodeling-related-BRCA1	RSmad	HBO1
pRb2/p130-multimolecular_complex	BRM-associated	SMAD3-E2F4/5-p107-DP1	ING5
SMAD3-cSKI-SIN3A-HDAC1	BRG1-associated	SMAD3-HEF1-APC10-CDH1	ING4
BRG1-SIN3A	EBAFa,b	SMAD3-SMAD4-FOXO3-FOXG1	hPRC1L
CoREST-HDAC	WINAC	SMAD3-SMAD4-cJun-cFos	KCNQ1_macromolecular
BRAF53-BRCA2	NUMAC_complex	SMAD3/4-E2F4/5-p107-DP1	p32-CBF-DNA
BRM-SIN3A	**Polycomb**	**Others**	MLL1-WDR5
TIP5-DNMT-HDAC1	BCOR	Ubiquitin_E3_ligase	MNK1-eIF4F
anti-BHC110	CEN	SNARE_complex	MOF
LSD1	E2F-6	CRM1-RAN-PHAX-CBC_complex	
Mi-2/NuRD-MTA2	EED-EZH2	PGC-1-SRp40-SRp55-SRp75	
M2/NuRD-BCL6-MTA3	Polycomb_repressive_complex_1,2,3	WIP-WASp-actin-myosin-IIa	
LARC_complex		Mis12_centromere	

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Complexes Disrupted

HDAC-containing	Switch-involved	SMADS	Others
Anti-HDAC2	BAF/pBAF	JUND-FOSB-SMAD3-SMAD4	BRAF-RAF1-14-3-3
BRM-SIN3A-HDAC	p300-CBP-p270-SWI/SNF	PML-SMAD2/3-SARA	BRCC
BHC/BHC110	SWI-SNF_chromatin_remodeling-related-BRCA1	RSmad	HBO1
pRb2/p130-multimolecular_complex	BRM-associated	SMAD3-E2F4/5-p107-DP1	ING5
SMAD3-cSKI-SIN3A-HDAC1	BRG1-associated	SMAD3-HEF1-APC10-CDH1	ING4
BRG1-SIN3A	EBAFa,b	SMAD3-SMAD4-FOXO3-FOXG1	hPRC1L
CoREST-HDAC	WINAC	SMAD3-SMAD4-p300	KCNQ1_macromolecular
BRAF53-BRCA2	NUMAC	SMAD3-SMAD4-p300-CBP	p32-CBF-DNA
BRM-SIN3A	Polycomb	SMAD3-SMAD4-p300-CBP-DP1	MLL1-WDR5
TIP5-DNMT-HDAC1	BCOR	SMAD3-SMAD4-p300-CBP-RNAPII	MNK1-eIF4F
anti-BHC110	CEN	SMAD3-SMAD4-p300-CBP-SWI/SNF	MOF
LSD1	E2F-6	SMAD3-SMAD4-p300-CBP-SWI/SNF-RNAPII	
Mi-2/NuRD-MTA2	EED-EZH2	SMAD3-SMAD4-p300-CBP-SWI/SNF-RNAPII-HNF4	
Mi2/NuRD-BCL6-MTA3	Polycomb_repressive_complex_1,2,3	WIP-WASp-actin-myosin-IIa	
LARC_complex		Mis12_centromere	

BHC controls expression of neuron-specific genes and neuronal differentiation. The component that is miRNA repressed, PHF21A, is enriched in differentiating ES cells. Its repression is speculated to have the opposite effect - that is, maintain the cells in a non-differentiated state

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Complexes Disrupted

HDAC-containing	Switch-involved	SMADS	Others
Anti-HDAC2	BAF/pBAF	JUND-FOSB-SMAD3-SMAD4	BRAF-RAF1-14-3-3
BRM-SIN3A-HDAC	p300-CBP-p270-SWI/SNF	PML-SMAD2/3-SARA	BRCC
BHC/BHC110	SWI-SNF_chromatin_remodeling-related-BRCA1	RSmad	HBO1
pRb2/p130-multimolecular_complex	BRM-associated	SMAD3-E2F4/5-p107-DP1	ING5
SMAD3-cSKI-SIN3A-HDAC1	BRG1-associated	SMAD3-HEF1-APC10-CDH1	ING4
BRG1-SIN3A	EBAFa,b	SMAD3-SMAD4-FOXO3-FOXG1	hPRC1L
CoREST-HDAC	WINAC	SMAD3-SMAD4-p300	KCNQ1_macromolecular
BRAF53-BRCA2	NUMAC	SMAD3-SMAD4-p300-CBP-DP1	p32-CBF-DNA
BRM-SIN3A	Polycomb	SMAD3-SMAD4-p300-CBP-RNAPII	MLL1-WDR5
TIP5-DNMT-HDAC1	BCOR	SMAD3-SMAD4-p300-CBP-SWI/SNF	MNK1-eIF4F
anti-BHC110	CEN	SMAD3-SMAD4-p300-CBP-SWI/SNF-RNAPII	MOF
LSD1	E2F-6	SMAD3-SMAD4-p300-CBP-SWI/SNF-RNAPII-HNF4	
Mi-2/NuRD-MTA2	EED-EZH2	SMAD3-SMAD4-p300-CBP-SWI/SNF-RNAPII-HNF4-HNF6	
Mi2/NuRD-BCL6-MTA3	Polycomb_repressive_complex_1,2,3	WIP-WASp-actin-myosin-IIa	
LARC_complex		Mis12_centromere	

The component in HPRC1L that is miRNA repressed (E3 ubiquitin-protein ligase RING2) is enriched in differentiating ES cells. Its repression is speculated to have opposite effect - that is, maintain the cells in a non-differentiated state

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Complexes Disrupted

HDAC-containing	Switch-involved	SMADS	Others
Anti-HDAC2	BAF/pBAF	JUND-FOSB-SMAD3-SMAD4	BRAF-RAF1-14-3-3
BRM-SIN3A-HDAC	p300-CBP-p270-SWI/SNF	PML-SMAD2/3-SARA	BRCC
BHC/BHC110	SWI-SNF_chromatin_remodeling-related-BRCA1	RSmad	HBO1
pRb2/p130-multimolecular_complex	BRM-associated	SMAD3-E2F4/5-p107-DP1	ING5
SMAD3-cSKI-SIN3A-HDAC1	BRG1-associated	SMAD3-HEF1-APC10-CDH1	ING4
BRG1-SIN3A	EBAFa,b	SMAD3-SMAD4-FOXO3-FOXG1	hPRC1L
CoREST-HDAC	WINAC	SMAD3-SMAD4-p300	KCNQ1_macromolecular
BRAF53-BRCA2	NUMAC	SMAD3-SMAD4-p300-CBP-DP1	p32-CBF-DNA
BRM-SIN3A	Polycomb	SMAD3-SMAD4-p300-CBP-RNAPII	MLL1-WDR5
TIP5-DNMT-HDAC1	BCOR	SMAD3-SMAD4-p300-CBP-SWI/SNF	MNK1-eIF4F
anti-BHC110	CEN	SMAD3-SMAD4-p300-CBP-SWI/SNF-RNAPII	MOF
LSD1	E2F-6	CRM1-RAN-PHAX-CBC_complex	
Mi-2/NuRD-MTA2	EED-EZH2	PGC-1-SRp40-SRp55-SRp75	
Mi2/NuRD-BCL6-MTA3	Polycomb_repressive_complex_1,2,3	WIP-WASp-actin-myosin-IIa	
LARC_complex		Mis12_centromere	

npBAF complexes which contain ACTL6A/BAF53A and PHF10/BAF45A, are exchanged for homologous alternative ACTL6B/BAF53B and DPF1/BAF45B or DPF3/BAF45C subunits in neuron-specific complexes (nBAF). DPF1 is miRNA targeted.

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Conclusions

- **miRNAs play impt role regulating formation of complexes**
  - Anti-coexpressed miRNAs tend to regulate direct partners of hubs
  - MiRNA disruption of complexes is a controlled and specific event
- **In VPA-treated mice, miRNAs disrupt neuron-specific and neuron-differentiating complexes**
- **The small # of disrupted complexes, and their precise roles, reaffirms miRNA action as precise**

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Acknowledgements

- **Wilson Goh**
- **Guimei Liu**
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