

Global and local topological properties of genome-wide regulatory network of *D. melanogaster*

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INTRODUCTION

Gene expression in higher eukaryotes is regulated at both the transcriptional and post-transcriptional levels. To obtain comprehensive understanding of the topological properties of the regulatory network, we combined transcriptional (ChIP-based) and post-transcriptional (miRNAs) regulations in *D. melanogaster* to create its genome-wide regulatory network. We used evolutionarily conserved instances of known sequence-specific motifs of both transcription factors (TFs) and miRNAs in 500± bp from the transcription start site (TSS) of every gene. To identify regulatory edges from TFs to miRNAs, we sought instances of sequence-specific motifs of TFs to experimentally determined promoters of the miRNA precursors (pri-mir). The overall network contains experimentally-derived binding profiles of 76 TFs, and genome-wide occurrences of 77 distinct evolutionarily-conserved miRNA seed motifs for 105 microRNAs.

NETWORK OVERVIEW

Quantity	ChIP+miRNA Network
TFs	76
miRNAs	105 (77)
TGs	12,680
TF to TG (TF) regulations	158,469 (1,847)
TF to miRNA regulations	89
miRNA to TG (TF) regulations	5,805 (106)
Diameter	5
Characteristic Path Length	2.11
No of Conn. Comp.	1
Clustering Coeff.	0.543
Density	0.16
Avg. In Degree - TF (miRNA)	12.50 (0.46)
Avg. Out Degree - TF (miRNA)	2,085 (75)

GLOBAL VIEW

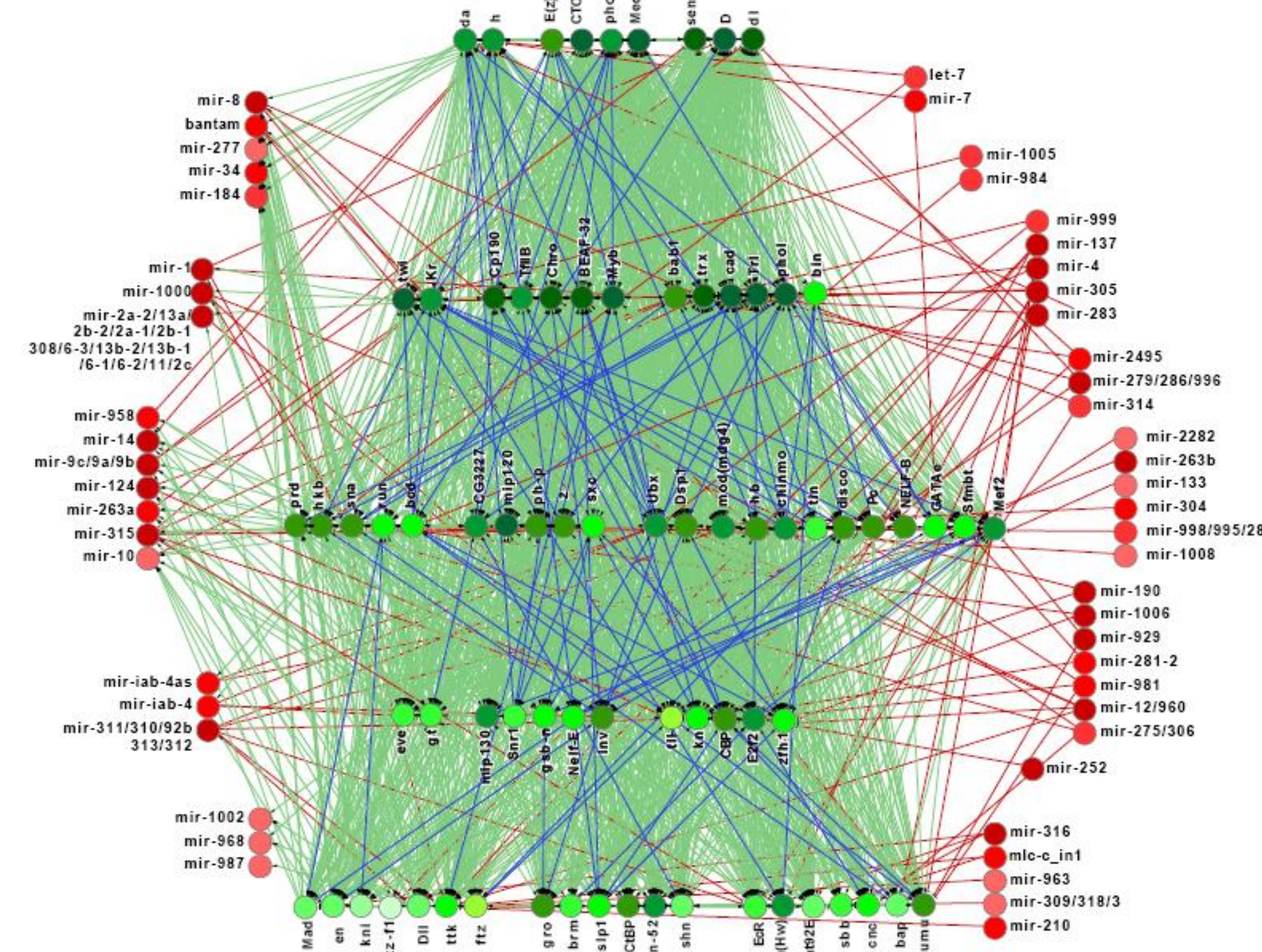


Figure 1. Hierarchical view of mixed regulatory network.

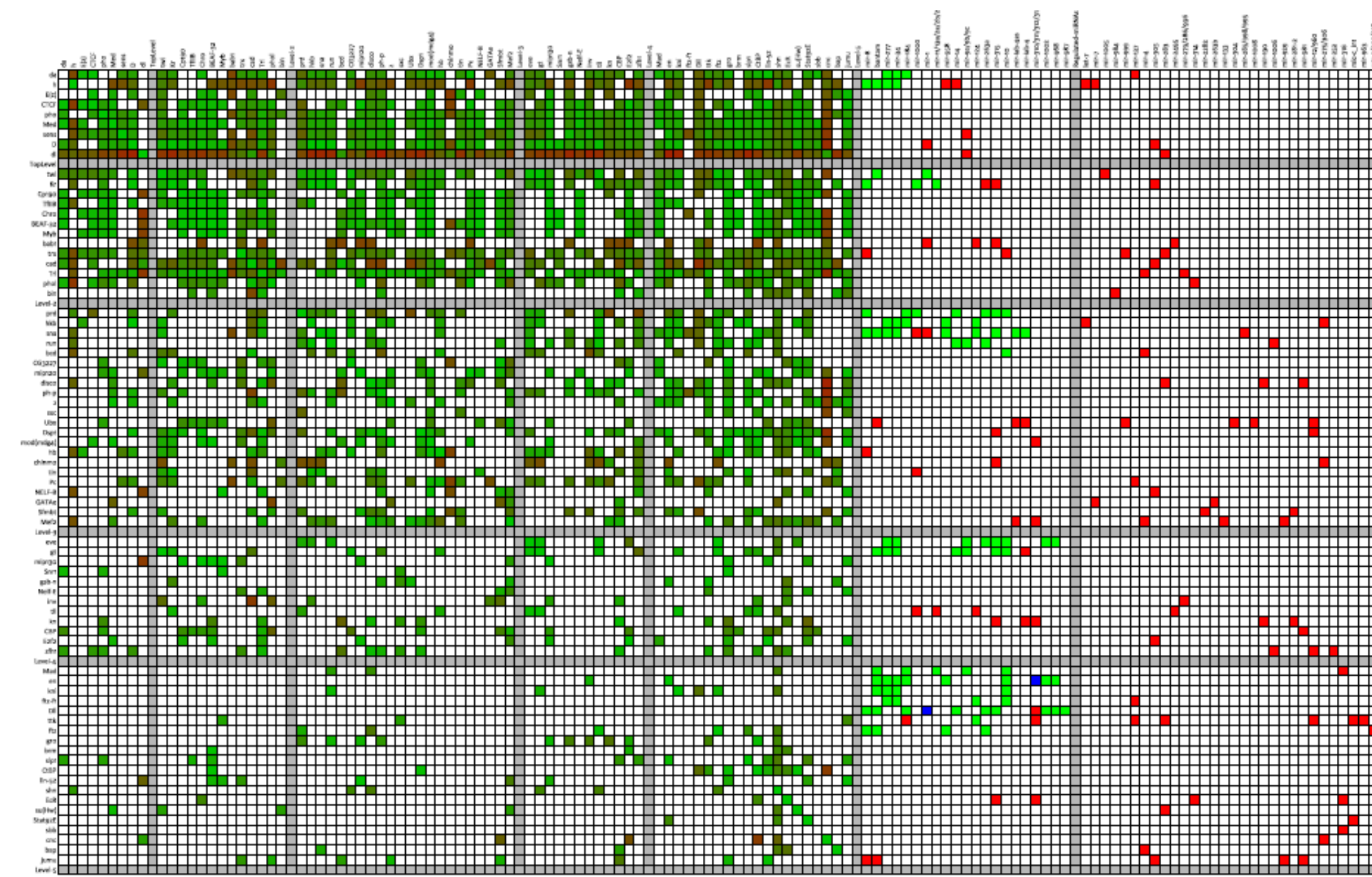


Figure 2. Heatmap of TF-TF, miRNA-TF and TF-miRNA regulations.

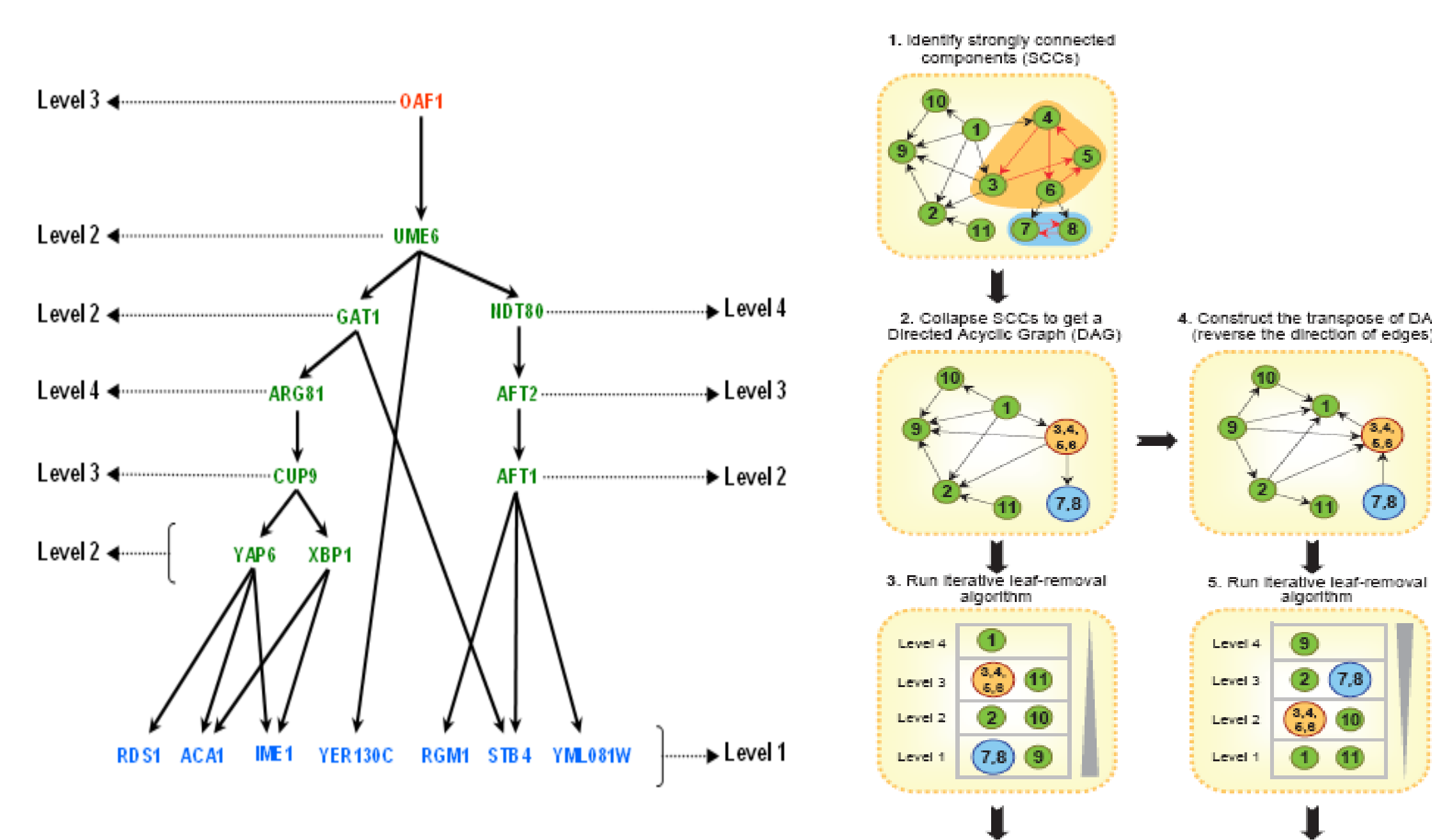


Figure 3. Bottom-up breadth first search strategy proposed for hierarchical decomposition [5].

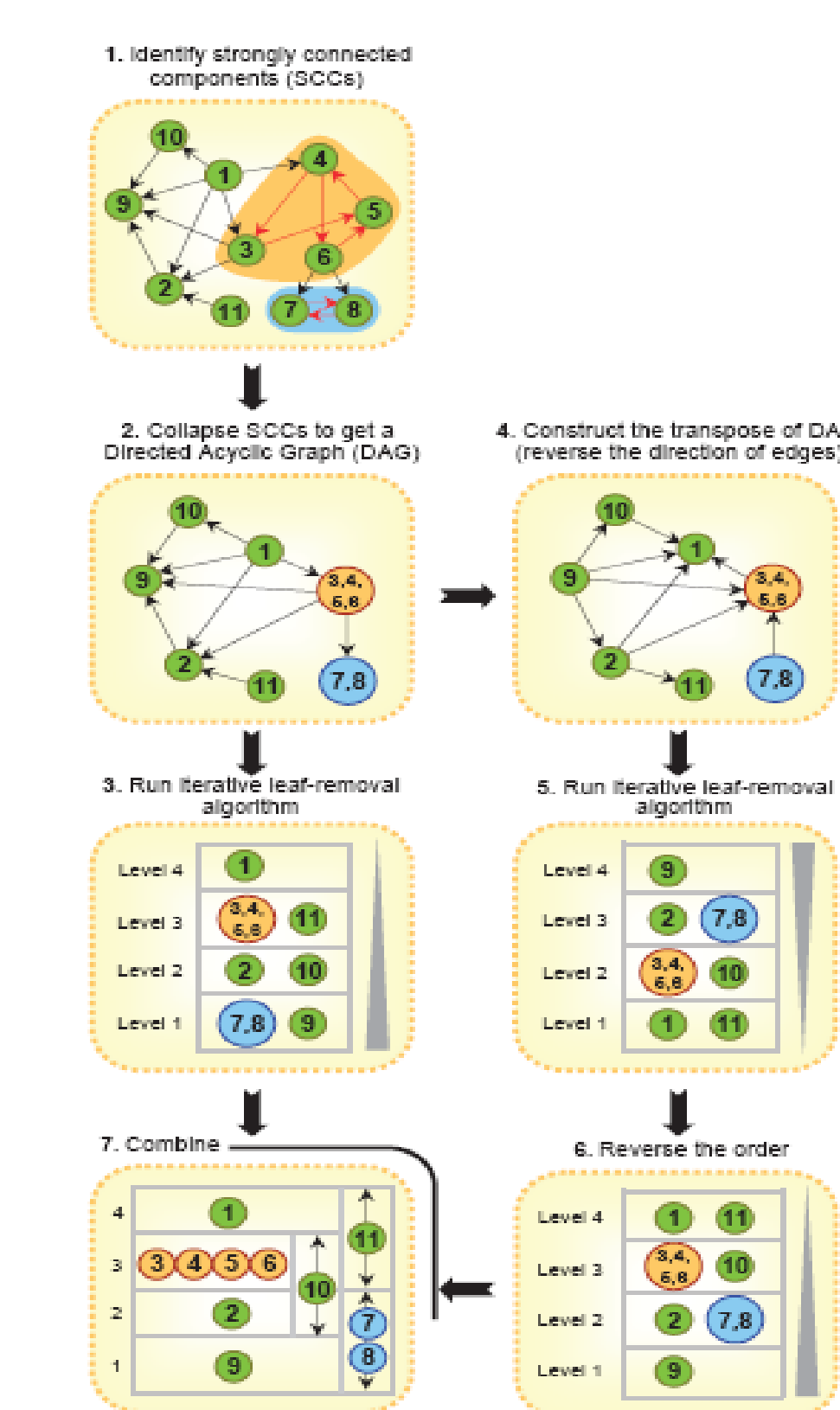


Figure 4. The vertex-sort algorithm [4].

Expression Characteristics of TFs

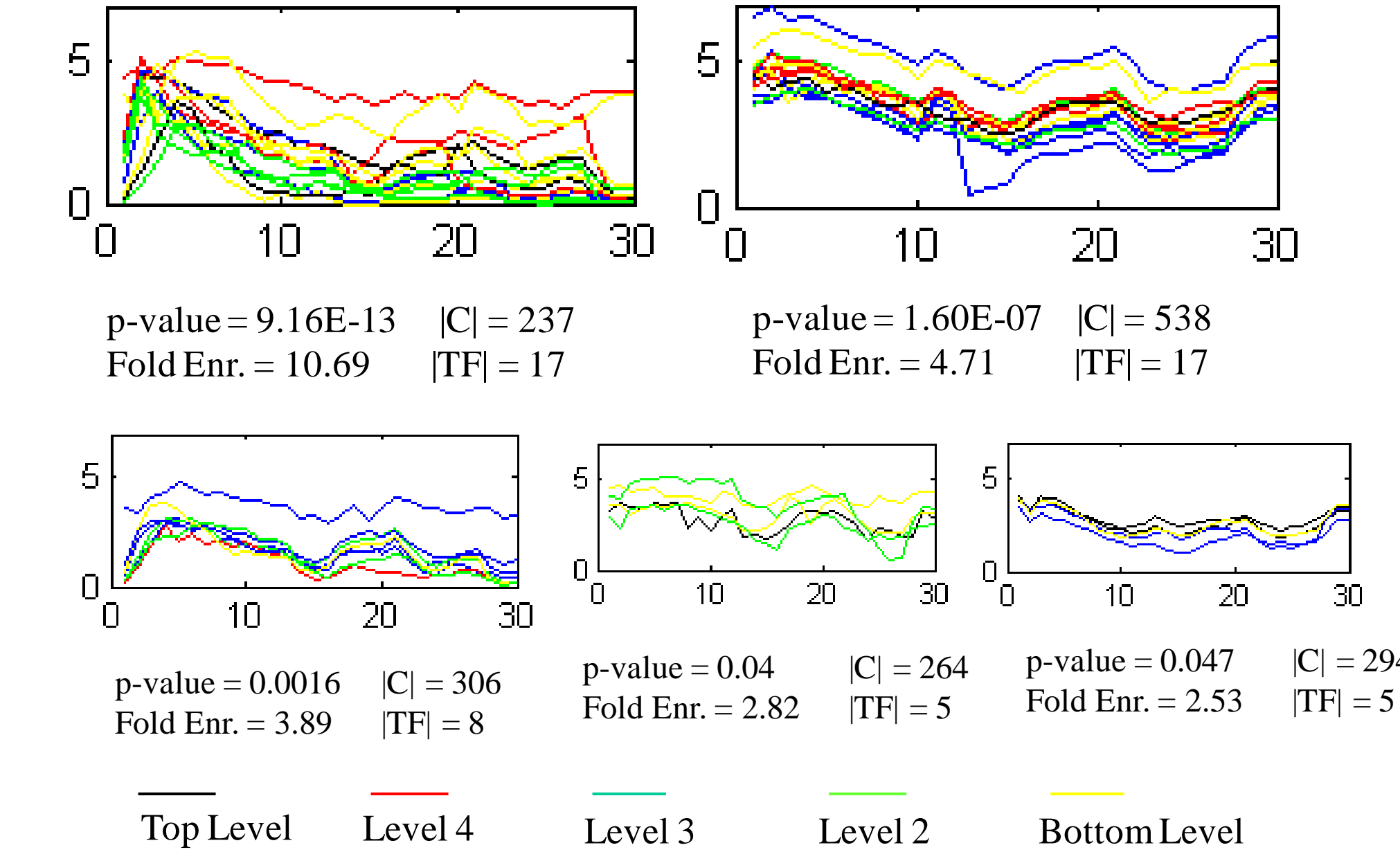


Figure 5. Expression clusters of developmental time course that are enriched in TFs.

NETWORK MOTIFS

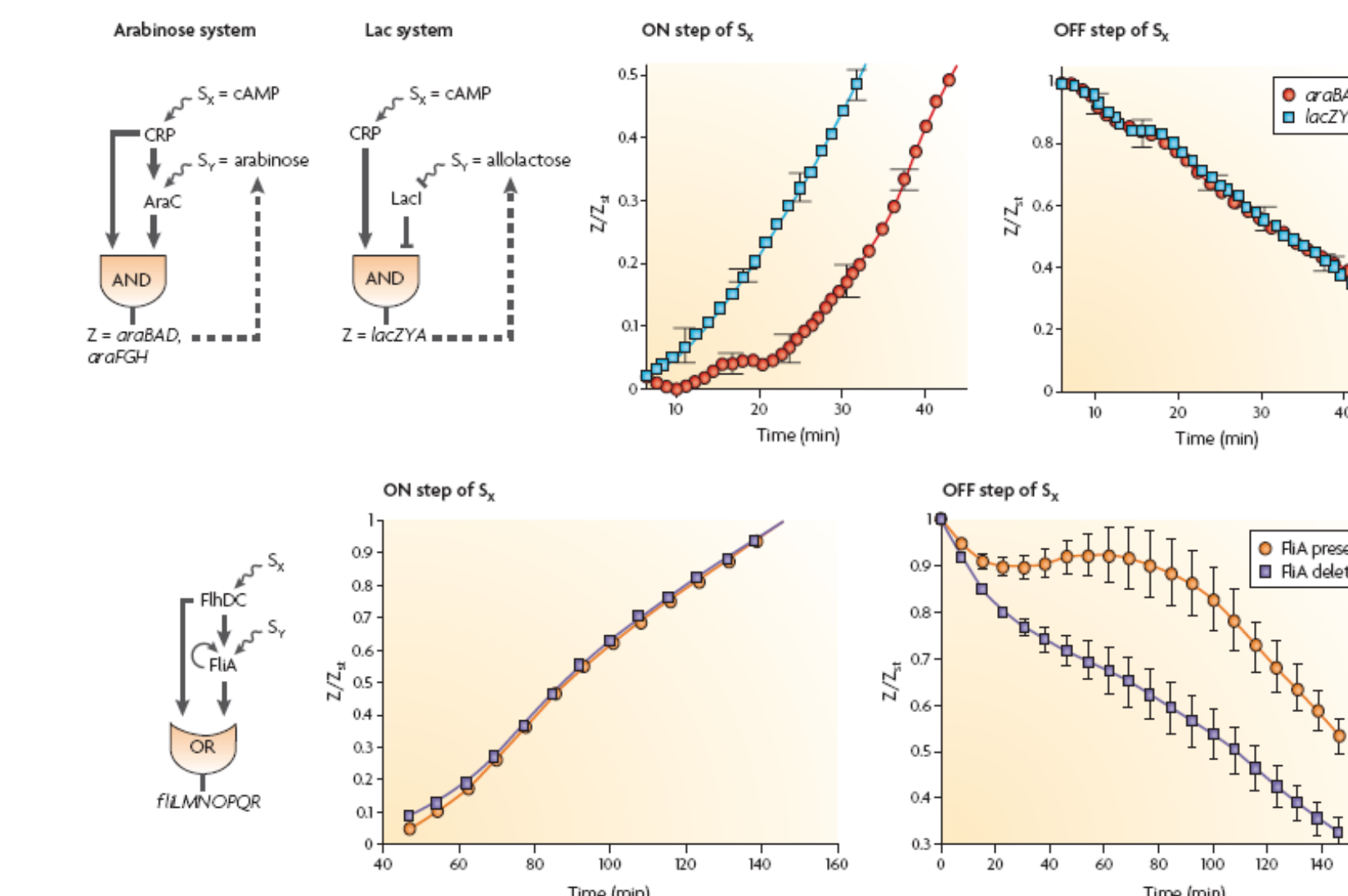


Figure 6. Coherent feed-forward loops and their dynamics. (Figure taken from Uri Alon's review in Nature Rev. Genetics 8, 2007[2])

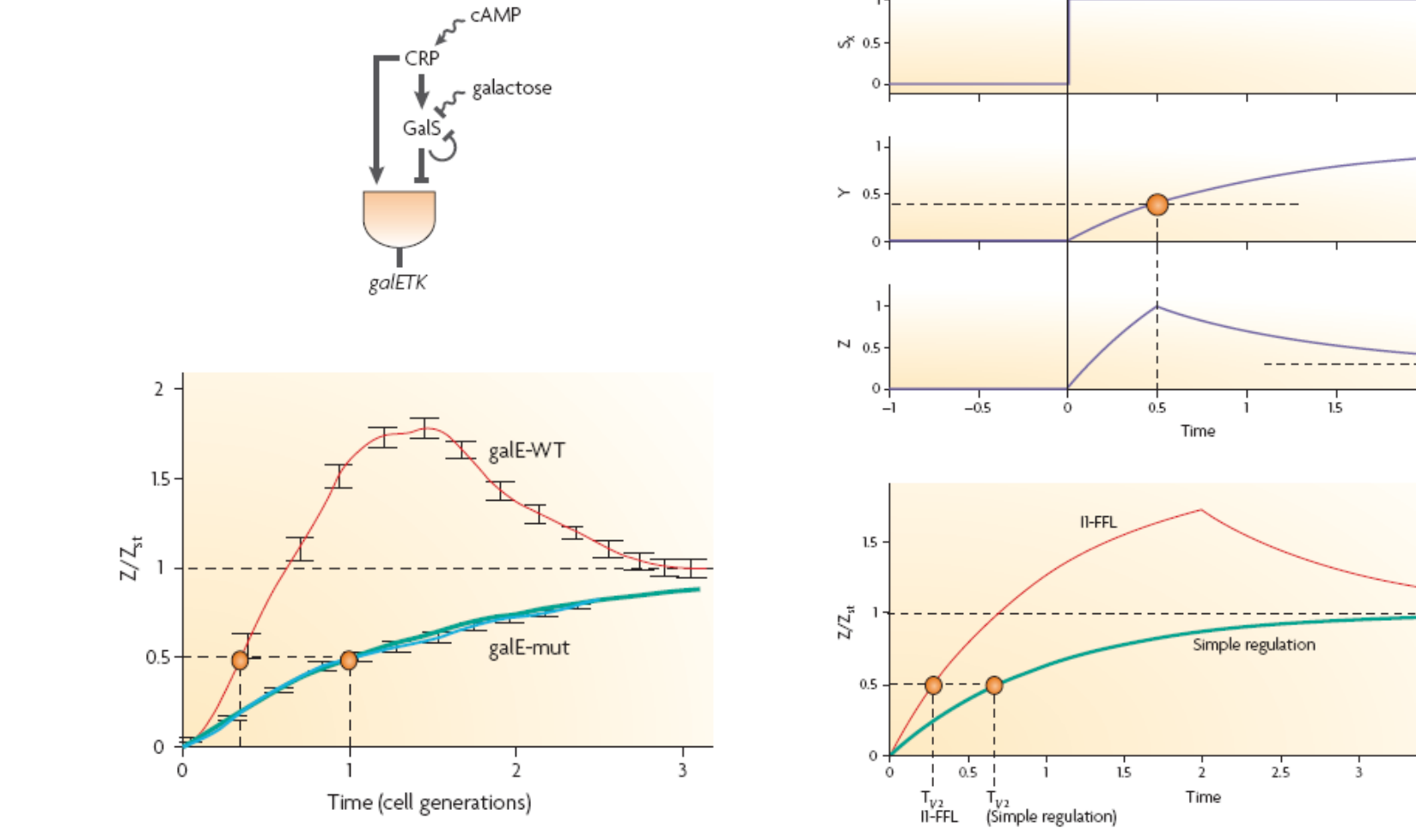


Figure 7. An incoherent feed-forward loop and its dynamics. (Figure taken from Uri Alon's review in Nature Rev. Genetics 8, 2007[2])

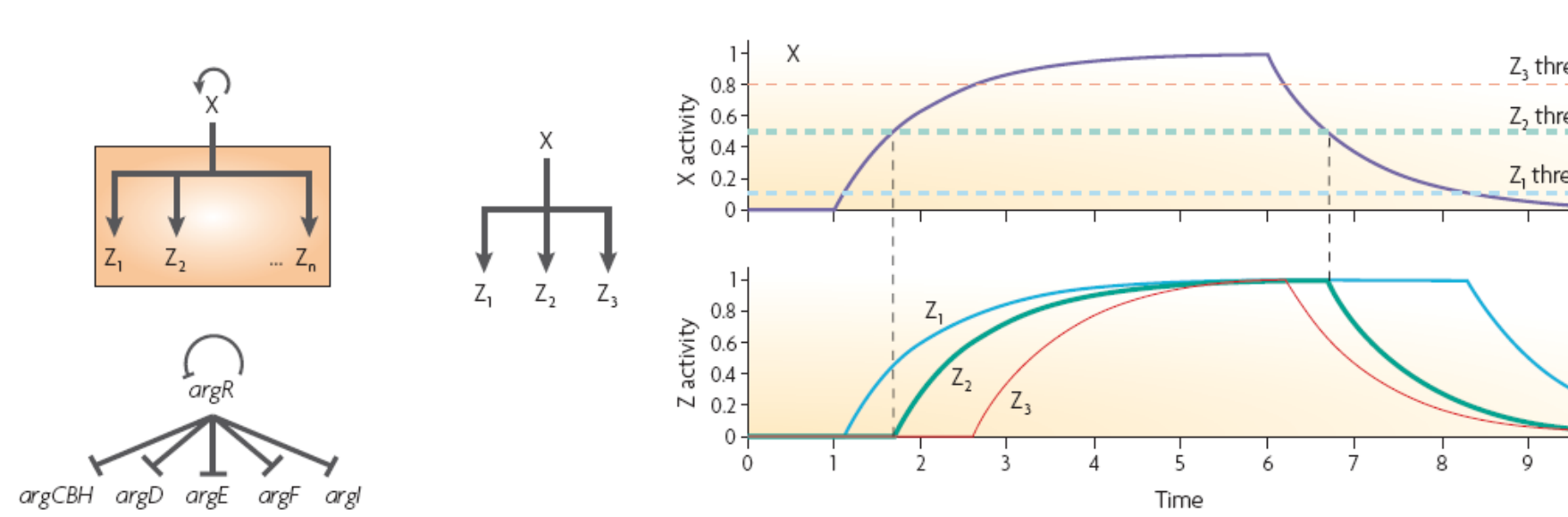


Figure 8. The single-input module network motif and its dynamics. (Figure taken from Uri Alon's review in Nature Rev. Genetics 8, 2007[2])

Description	Illustration	Statistical Significance		Examples (Activator, Repressor)		
		Fold Enr.	Z-score	A	B	C
Cross-regulating TFs co-targeting a miRNA	1	2.996	5.637	twi sna eve run kni	h prd gt prd gt	bantam mir-8 mir-10 mir-14 mir-277
Cross-regulatory clique of TFs	2	2.063	3.453	bed mod(mdg4) BEAF-32 Cp190 cad	Kr Myb Mip120 Chro	pb-p Dsp1 Med phol dl
Feed-forward loop with cross-regulating TFs and a miRNA	3	1.969	2.311	mir-1 mir-315 mir-14 mir-263a mir-8	twi gt run prd h	sna Kr h Kr hb
Cross-regulating TFs co-targeted by another TF	4	1.294	4.507	prl bab1 TFIIIB da GAT4e	gt trx mip130 Mef2 Mef2	shn disco Mycb lin-52 z
Feedback loop with a miRNA	5	1.273	1.295	mir-1000 mir-1 mir-315 mir-8 mir-9a/9b/9c	Kr C15 sna nub eve	tin sna Kr hb seis
Feed-forward loop with a miRNA ending at a target gene	6	1.259	1.797	mir-958 bantam mir-8 mir-124 mir-263a	hkb twi erb sna run	Cak dap erf Gfi Mes2
Cross-regulating TFs co-targeting a target gene	7	1.256	12.625	pho D cll phol mip120	kn da lin-52 Med Myb	Kesap1 dnk gr tna Run
Cross-regulating TFs co-targeting another TF	8	1.158	7.117	z Dsp1 gt trx prd	phol sha disco ph-p	lin-52 cie CBP Antp

Table 2. Highly-enriched network motifs in mixed regulatory network. Green nodes represent TFs, red nodes represent miRNAs and black nodes represent target genes.

- Extensive co-binding and co-targeting of TFs.
 - ❑ Five motifs (1, 2, 4, 7 and 8) exhibit patterns of TF co-operation.
- Enrichment of positive and negative feedbacks.
 - ❑ Due to cross-regulation between two or more TFs in motifs 1, 2, 3, 4, 7 and 8.
- Tuning of expression delay properties by post-transcriptional regulation.
 - ❑ Mixed feed-forward loops including miRNAs in motifs 3 and 6
- Feedback from lower level TFs to upper level TFs (consistent with the hierarchical structure in Figure 1).
 - ❑ Feedback loop between two TFs using a miRNA in motif 5.
- Specific instances of each motif should be further evaluated experimentally to understand its biological meaning.
 - ❑ E.g. The transcription factors *SNAIL*, *TWIST* and the miRNA *mir-1* are all known to be co-expressed mesodermally. Furthermore, *TWIST* is a known activator for both *SNAIL* and *mir-1* [6].

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