Enrichment and aggregation of topological motifs are independent organizational principles of integrated interaction networks: Supplementary Tables

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I. FUNCTIONAL CATEGORIES ENRICHED IN TRANSCRIPTIONAL FEEDFORWARD LOOP CLUSTERS

MIPS functional category	Enriched clusters
Regulation of amino acid metabolism	31, 66
Regulation of nitrogen, sulfur and selenium metabolism	31
C-compound and carbohydrate metabolism	2, 25, 41
Phospholipid metabolism	43
Regulation of lipid, fatty acid and isoprenoid metabolism	31, 55
Metabolism of energy reserves (e.g. glycogen, trehalose)	4
Cytokinesis (cell division) /septum formation and hydrolysis	20
General transcription activities	6, 37, 43, 45, 66
Transcriptional control	1, 6, 38, 47, 50, 56, 73, 78, 79
Transcription activation	31
Ribosomal proteins	54
Protein binding	79
DNA binding	16, 31, 55, 64
Sugar transport	2, 6, 10
Cellular import	2
Stress response	51
Pheromone response	51, 79
Mating (fertilization)	1

TABLE S1 MIPS functional categories enriched with hypergeometric $P < 10^{-3}$ in clusters containing at least four transcriptional regulatory feedforward loop instances and with at least three proteins annotated to the enriched category.

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II. TRANSCRIPTIONAL REGULONIC PROTEIN COMPLEXES

Cluster ID	Regulator	♯ Nodes	Int. density	Protein complex	P-value
1	RAP1,FHL1	27	0.79	Ribosomal large subunit	8×10^{-12}
				Ribosomal small subunit	2×10^{-11}
2	YAP5,GAT3	10	0.8	Ribosomal large subunit	7×10^{-8}
				Ribosomal small subunit	1×10^{-8}
3	SPT2,GCR2,AZF1	7	0.95	Ribosomal large subunit	1×10^{-12}
5	PDR1	7	0.81	Ribosomal large subunit	9×10^{-7}
				Ribosomal small subunit	2×10^{-5}
6	HIR1,HIR2	7	0.71	Nucleosomal protein complex	7×10^{-12}
10	SWI4	6	0.73	Nucleosomal protein complex	2×10^{-5}
12	AZF1	8	0.75	Ribosomal large subunit	0
14	ABF1	5	1	Mit. ribosomal large subunit	2×10^{-11}
22	MET31	4	1	Ribosomal large subunit	8×10^{-6}
26	ZAP1	4	1	Ribosomal large subunit	8×10^{-6}
29	SFP1	4	1	Ribosomal small subunit	6×10^{-9}
65	PHO2	3	1	SAGA complex	1×10^{-8}
124	RAP1,FHL1	3	1	Ribosomal large subunit	2×10^{-6}

TABLE S2 Transcriptionally coregulated interacting protein clusters with the topology of a regulonic complex, defined as a protein subnetwork with at least 3 nodes, at least 70% of all possible pairwise interactions present, and a hypergeometric overlap P-value with a protein complex smaller than 10^{-4}.

III. FUNCTIONAL ANNOTATION OF ABF1 USING COREGULATED PPI SUBNETWORKS

Cluster ID	MIPS functional category	P-value
11	tRNA synthesis	2×10^{-10}
	rRNA synthesis	2×10^{-9}
	rRNA processing	8×10^{-8}
	DNA binding	3×10^{-5}
	RNA binding	7×10^{-5}
14	Mitochondrion	1×10^{-8}
	Ribosomal proteins	9×10^{-8}
15	RNA degradation	3×10^{-6}
	Splicing	5×10^{-6}
21	Cytoskeleton-dependent transport	7×10^{-5}
25	Nuclear transport	7×10^{-7}
	RNA transport	3×10^{-5}
31	Vesicle fusion	5×10^{-6}
	ER to Golgi transport	4×10^{-5}
71	DNA binding	5×10^{-4}
90	Protein folding and stabilization	3×10^{-5}
	Protein targeting, sorting and translocation	8×10^{-4}
91	rRNA processing	7×10^{-6}
105	rRNA processing	1×10^{-4}

TABLE S3 Functional categories enriched with P-value $< 10^{-3}$ in transcriptionally coregulated interacting protein clusters regulated by ABF1. Only categories with at least three proteins in the cluster annotated to it are included.

IV. FUNCTIONAL CATEGORIES ENRICHED IN POSTTRANSLATIONAL FEEDFORWARD LOOP CLUSTERS CONTROLLED BY CDC28 OR PHO85

MIPS functional category	Enriched clusters
Metabolism of glutamate	1
Phosphate metabolism	7, 9, 12, 22
DNA synthesis and replication	14
G1/S transition of mitotic cell cycle	7
G2/M transition of mitotic cell cycle	9, 38
M phase	9
Meiosis	12
Transcriptional control	16
Modification by (de-)(auto-)phosphorylation	7, 8, 9, 12, 16, 22, 38, 40
Cyclic nucleotide binding (cAMP, cGMP, etc.)	16
Enzymatic activity regulation / enzyme regulator	33
Nutrient starvation response	8
Transposable elements, viral and plasmid proteins	3
Cell growth / morphogenesis	25, 38
Cytoskeleton/structural proteins	25
Budding, cell polarity and filament formation	5, 7, 25, 38

TABLE S4 MIPS functional categories enriched with hypergeometric $P < 10^{-3}$ in clusters containing at least four posttranslational regulatory feedforward loop instances, with at least three proteins annotated to the enriched category, and controlled by CDC28 or PHO85.

MIPS functional category	Enriched clusters
Phosphate metabolism	3, 10
Regulation of phosphate metabolism	4, 7
DNA synthesis and replication	1
Mitotic cell cycle	1
G1 phase of mitotic cell cycle	12
G1/S transition of mitotic cell cycle	1, 27
G2/M transition of mitotic cell cycle	1, 8, 13, 14, 23
General transcription activities	1
Transcriptional control	4
Transcription activation	1
Modification by (de-)(auto-)phosphorylation	3, 4, 10, 15
Enzymatic activity regulation / enzyme regulator	1
Ca2+ mediated signal transduction	3
Nutrient starvation response	4
Perception of nutrients and nutritional adaptation	15
Cell growth / morphogenesis	1
Cytoskeleton/structural proteins	1
Budding, cell polarity and filament formation	1, 13

TABLE S5 MIPS functional categories enriched with hypergeometric $P < 10^{-3}$ in clusters containing at least four mixed posttranslational-transcriptional regulatory feedforward loop instances, with at least three proteins annotated to the enriched category, and controlled by CDC28 or PHO85.

V. TRANSCRIPTIONAL AND POSTTRANSLATIONAL PROTEIN-INTERACTION MEDIATED REGULONIC STARS

Cluster ID	Regulator	Hub	♯ Spokes	MIPS functional category	P-value
5	ABF1	SMT3	17	DNA binding	8×10^{-5}
9	RAP1	SMT3	11		
11	ABF1	PSE1	10	Nuclear transport	3×10^{-7}
14	HAP1	HSP82	9		
16	ABF1	GLC7	8		
21	FHL1	HMO1	6		
28	SPT2	CKA1	5	DNA binding	5×10^{-4}
29	RAP1	TAF1	5	DNA conformation modification	2×10^{-5}
32	MBP1	RAD53	5	Cell cycle checkpoints	4×10^{-5}
36	FKH1	NOP1	5	rRNA processing	6×10^{-4}
44	ABF1	CKA1	4		

TABLE S6 Transcriptional protein-interaction mediated regulatory loop clusters with the topology of a regulonic star. ♯ **Spokes** is the number of spoke proteins interacting with the **Regulator** and **Hub** protein. **MIPS functional category** is the most enriched functional category, only shown if it has **P-value** $< 10^{-3}$ and at least three proteins in the cluster annotated to it.

Cluster ID	Regulator	Hub	♯ Spokes	MIPS functional category	P-value
10	YCK1	HSP82	19		
38	HMT1	NPL3	8	RNA binding	2×10^{-9}
43	CLB2	NAP1	7	Budding, cell polarity and filament formation	6×10^{-4}
61	SNF1	SMT3	6		
62	CLB2	KEL1	8	Budding, cell polarity and filament formation	3×10^{-4}
63	PHO85	CLB2	6		
64	PHO85	YKU80	8	Regulation of phosphate metabolism	2×10^{-6}
66	YPL141C	HSP82	6		
67	KSP1	SRS2	6		
77	IPL1	YRA1	5		
99	CMK1	CMD1	5		
103	CDC28	RPO21	4		
118	SNF1	GLC7	4		
119	PHO85	YHB1	4		
120	ATG1	ATG17	4		
121	MEC1	RAD53	4	Cell cycle checkpoints	2×10^{-7}
122	PCL1	PHO4	4		

TABLE S7 Posttranslational protein-interaction mediated regulatory loop clusters with the topology of a regulonic star. ♯ **Spokes** is the number of spoke proteins interacting with the **Regulator** and **Hub** protein. **MIPS functional category** is the most enriched functional category, only shown if it has **P-value** $< 10^{-3}$ and at least three proteins in the cluster annotated to it.

VI. PROTEIN COMPLEXES FORMING PROTEIN-INTERACTION MEDIATED TRANSCRIPTIONAL REGULATORY INTERACTING DOUBLE-STARS

Cluster ID	Regulator	Hub	♯ Spokes	Protein complex name	P-value
1	YAP1	SRB6, SRB7	25	Srb10p complex	2×10^{-7}
1	YAP1	SRB6, SRB7	25	SAGA complex	4×10^{-7}
1	YAP1	SRB6, SRB7	25	SAGA-like complex	6×10^{-7}
1	YAP1	SRB6, SRB7	25	SRB Mediator complex	1×10^{-12}
4	PHO4	RPS1B	18	Ribosomal large subunit	4×10^{-12}
4	PHO4	RPS1B	18	Ribosomal small subunit	5×10^{-7}
15	REB1	STH1	8	RSC complex	8×10^{-5}
17	ADR1	TAF3, MED1	7	ADA complex	3×10^{-5}
17	ADR1	TAF3, MED1	7	SAGA complex	2×10^{-11}
17	ADR1	TAF3, MED1	7	SAGA-like complex	2×10^{-5}
17	ADR1	TAF3, MED1	7	TAFIIs	1×10^{-12}
19	YAP1	SSN8	7	Srb10p complex	8×10^{-12}
19	YAP1	SSN8	7	SRB Mediator complex	6×10^{-9}
20	HAP4, KSS1	GCN4	5	SWI/SNF complex	4×10^{-12}
23	SNF1	SRB6, NUT2	5	Srb10p complex	6×10^{-6}
23	SNF1	SRB6, NUT2	5	SRB Mediator complex	1×10^{-6}
33	FKH1, FKH2	HHF1, FKH1	5	Nucleosomal protein complex	3×10^{-6}
47	HIR1	MAM33	3	Nucleosomal protein complex	8×10^{-6}
49	KSS1	GCN4	3	19/22S regulator	5×10^{-5}

TABLE S8 Protein-interaction mediated transcriptional regulatory loop clusters with the topology of a regulatory interacting double-star. All clusters with one or two regulators and hub proteins and at least 3 spoke proteins are shown if they have a hypergeometric overlap P-value with a protein complex smaller than 10^{-4}.

VII. PROTEIN COMPLEXES FORMING PROTEIN-INTERACTION MEDIATED POSTTRANSLATIONAL REGULATORY INTERACTING DOUBLE-STARS

Cluster ID	Regulator	Hub	♯ Spokes	Protein complex name	P-value
7	HRR25	ENP1,RPS3	18	Ribosomal small subunit	5×10^{-12}
9	PRP43	SPP382	21	mRNA splicing	1×10^{-7}
9	PRP43	SPP382	21	Prp19p-associated complex	3×10^{-5}
13	FIP1	PAP1	16	pre mRNA3′-end processing factor CFII	4×10^{-11}
13	FIP1	PAP1	16	pre mRNA polyadenylation factor PFI	2×10^{-5}
14	KIN28,MED6	RPO21	14	RNA polymerase II	3×10^{-6}
14	KIN28,MED6	RPO21	14	SRB Mediator complex	0
17	CKB2	HHF1,HHF2	11	Casein kinase II	2×10^{-5}
18	SLA1	LAS17	14	Actin-associated proteins	2×10^{-9}
24	PAB1	CRM1	12	Nuclear pore complex (NPC)	8×10^{-9}
24	PAB1	CRM1	12	NSP1 complex	2×10^{-5}
27	SIN3	HHT1,HHT2	10	Casein kinase II	2×10^{-8}
27	SIN3	HHT1,HHT2	10	Nucleosomal protein complex	5×10^{-10}
31	EPL1	HHF1,HHF2	7	Nucleosomal protein complex	5×10^{-8}
32	SSN3	MED2	9	SRB Mediator complex	6×10^{-11}
39	CDC28	SPC110	5	SPB components	1×10^{-7}
42	SMT3	CBF2	8	CBF3 protein complex	1×10^{-5}
47	COF1	ACT1	7	Actin-associated proteins	3×10^{-11}
50	FUS3,STE11	STE7	7	STE5-MAPK complex	6×10^{-12}
51	SIR2	HHF1	6	Nucleosomal protein complex	3×10^{-5}
51	SIR2	HHF1	6	RNA polymerase I	1×10^{-4}
52	RPO21	KIN28	7	TFIIH	5×10^{-5}
53	SNF1	GCN5	6	Serine/threonine phoshpoprotein phosphatase	2×10^{-8}
54	GCN5	HHT1,HTA1	5	Nucleosomal protein complex	3×10^{-5}
54	GCN5	HHT1,HTA1	5	SAGA-like complex (SLIK)	1×10^{-5}
56	PRK1,ARK1	SLA1,PAN1	3	Actin-associated proteins	1×10^{-11}
57	CTK1	HTZ1	7	Nucleosomal protein complex	6×10^{-12}
60	SSN3	GCN4	6	SRB Mediator complex	2×10^{-12}
71	GLC7	PTA1	6	cytoplasmic ribosomal large subunit	6×10^{-9}
80	CDC34,SIC1	CLN2	7	Cdc28p complexes	6×10^{-5}
80	CDC34,SIC1	CLN2	7	SCF-CDC4 complex	1×10^{-8}
80	CDC34,SIC1	CLN2	7	SCF-GRR1 complex	5×10^{-9}
80	CDC34,SIC1	CLN2	7	SCF-MET30 complex	8×10^{-6}
87	SSN8	GAL4	5	Srb10p complex	5×10^{-6}
88	RNA1	GSP1	5	Nuclear pore complex (NPC)	6×10^{-5}
89	KIN28	RGR1,MED4	4	RNA polymerase II	4×10^{-5}
89	KIN28	RGR1,MED4	4	SRB Mediator complex	3×10^{-7}
92	GIN4	SHS1	5	Septin filaments	4×10^{-12}
94	CDC9	RAD24	5	Replication factor C complex	2×10^{-11}
97	BBC1	LAS17	5	Actin-associated proteins	1×10^{-6}
97	BBC1	LAS17	5	Actin-associated motorproteins	2×10^{-5}
98	PHO81	PHO85	5	Pho85p complexes	1×10^{-5}
100	RTT109	HHT1,HHT2	4	Nucleosomal protein complex	8×10^{-6}
101	CDC28	RPN10	4	19/22S regulator	2×10^{-10}
102	CDC5	MCD1,REC8	3	Sister chromatid cohesion complex	2×10^{-11}
113	FUS3	STE5,HSC82	5	STE5-MAPK complex	8×10^{-9}
115	GLO3	ARF1	4	COPI	1×10^{-11}
116	CKA1	MIG1	4	Nucleosomal protein complex	1×10^{-8}
117	RPO21	SSL1	4	TFIIH	2×10^{-8}
117	RPO21	SSL1	4	NEF3 complex	2×10^{-5}
132	CDC7	MCM2,DBF4	4	Post-replication ORC complex	8×10^{-6}
132	CDC7	MCM2,DBF4	4	Replication initiation complex	1×10^{-8}
132	CDC7	MCM2,DBF4	4	Pre-replication complex (pre-RC)	1×10^{-7}
132	CDC7	MCM2,DBF4	4	Replication complex	3×10^{-7}
133	GLC7,PRK1	PAN1	4	Actin-associated proteins	5×10^{-7}

TABLE S9 Protein-interaction mediated posttranslational regulatory loop clusters with the topology of a regulatory interacting double-star. All clusters with one or two regulators and hub proteins and at least 4 spoke proteins are shown if they have a hypergeometric overlap P-value with a protein complex smaller than 10^{-4}.

VIII. KEY TO CLUSTERING ALGORITHM OUTPUT FILES

We supply as separate text files the output of the motif clustering algorithm for each of the 10 significantly aggregating network motifs. These files list for each cluster the motif instances assigned to it, and have the format

<div align="center">

ClusterID   GeneX   GeneY   GeneZ

</div>

The order of interactions can be deduced from the file name: T = transcriptional, B = biochemical (posttranslational and P = protein-protein interaction. In the file names below, the first letter refers to the interaction between GeneX and GeneY, the second between GeneY and GeneZ and the third between GeneZ and GeneX; 'i' refers to 'inverse', i.e. 'Ti' between Z and X means a transcriptional interaction from X to Z. For symmetric motifs (e.g. TBTi), each motif instance is listed twice, once with the PPI in each direction, since this is how the algorithm stores such motifs internally.

The result files together with the source code of the algorithm are available at

<div align="center">

http://omics.frias.uni-freiburg.de/motifclust

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Network motif	File name
Transcriptional feedforward loop	TTTi_clusters.txt
Transcriptionallly coregulated interacting proteins	TPTi_clusters.txt
Copointing interacting transcriptional regulators	TiPT_clusters.txt
Copointing interacting posttranslational regulators	BiPB_clusters.txt
Protein-interaction mediated transcriptional regulatory loop	TPP_clusters.txt
Protein-interaction mediated posttranslational regulatory loop	BPP_clusters.txt
Posttranslational feedforward loop	BBBi_clusters.txt
Mixed posttranslational-transcriptional feedforward loop	BTBi_clusters
Mixed posttranslational-transcriptional feedforward loop with PPI 1	PTBi_clusters.txt
Mixed posttranslational-transcriptional feedforward loop with PPI 2	TPB_clusters.txt

TABLE S10 Key to clustering algorithm output files for all significantly aggregating network motifs.