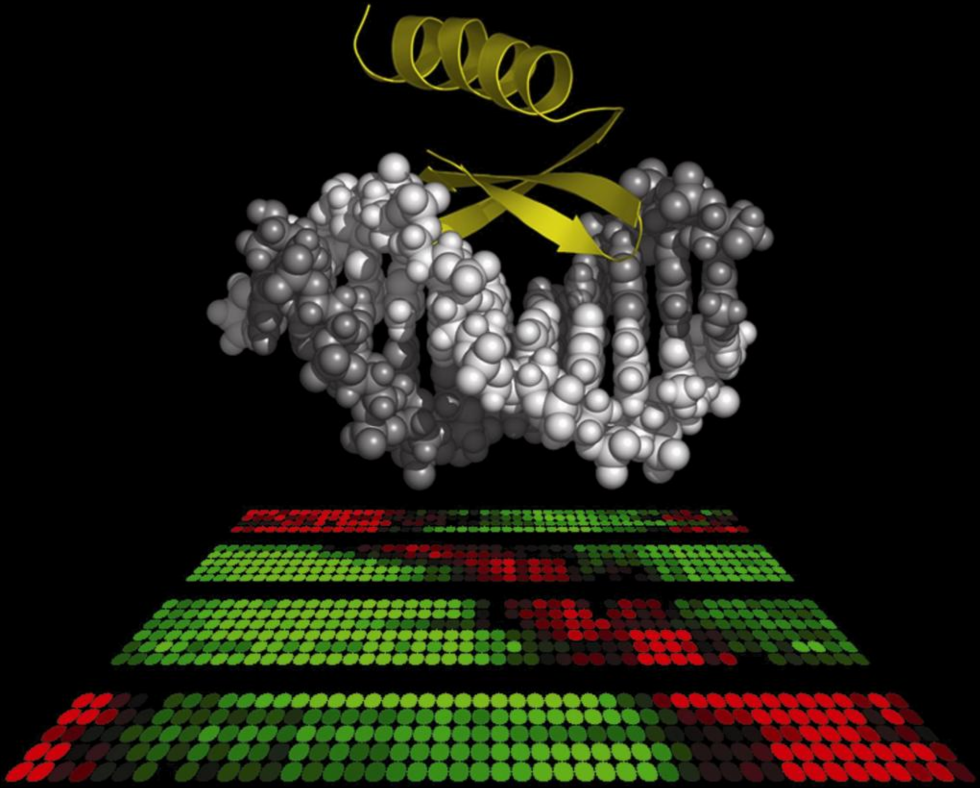
Structure, evolution and dynamics of gene regulatory networks



M. Madan Babu

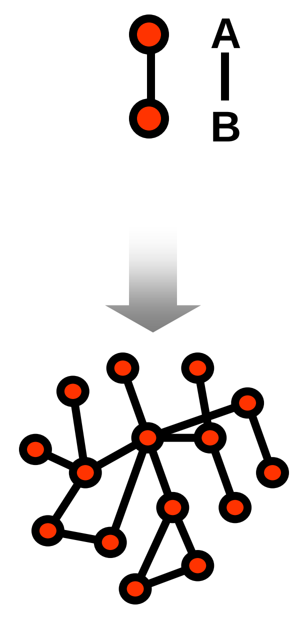
Group Leader

MRC Laboratory of Molecular Biology, Cambridge

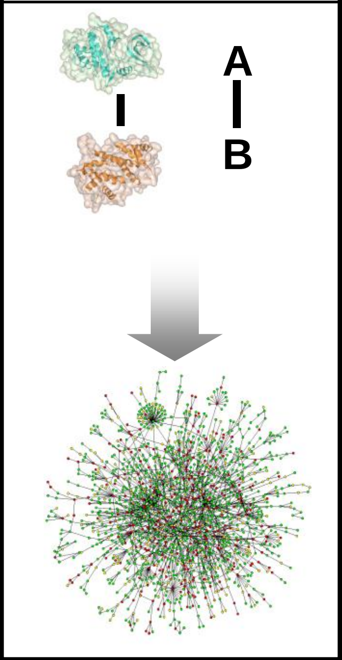
Networks in Biology

Network	**Protein Interaction**	**Metabolic**	**Transcriptional**
Nodes ●	*Proteins*	*Metabolites*	*Transcription factor* *Target genes*
Links ●–●	*Physical Interaction*	*Enzymatic conversion*	*Transcriptional Interaction*

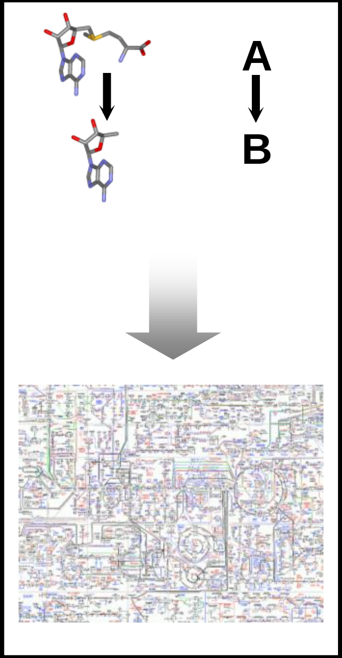
Interaction



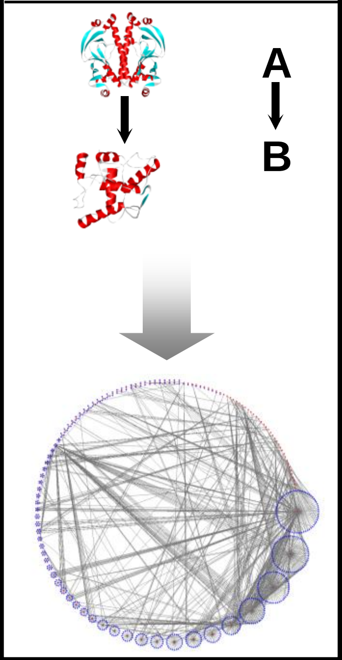
Protein-Protein



Protein-Metabolite



Protein-DNA

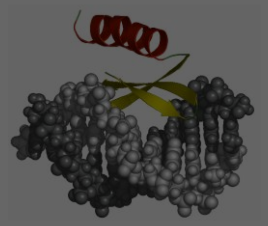


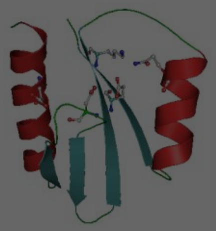
Overview: How is regulation achieved in cellular systems?

Scale of complexity

Molecular Level

Discover new features of regulatory systems

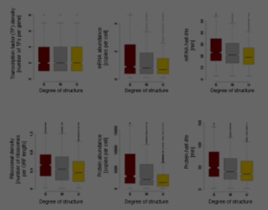

Nuc. Acids. Res (2006)

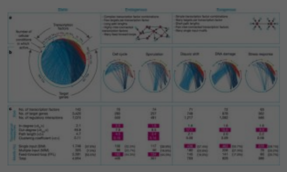

J Bacteriol (2008)

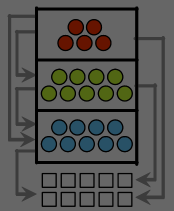
Molecular Cell (2008)

Systems Level

Principles of regulation for cellular homeostasis

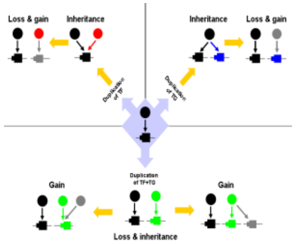

Science (2008)


Nature (2004)

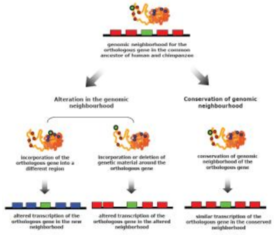

Mol Sys Bio (2009)

Genome Level

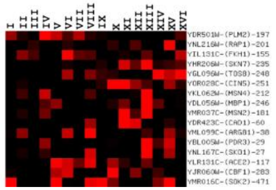
Regulation and genome evolution



Nature Genetics (2004)



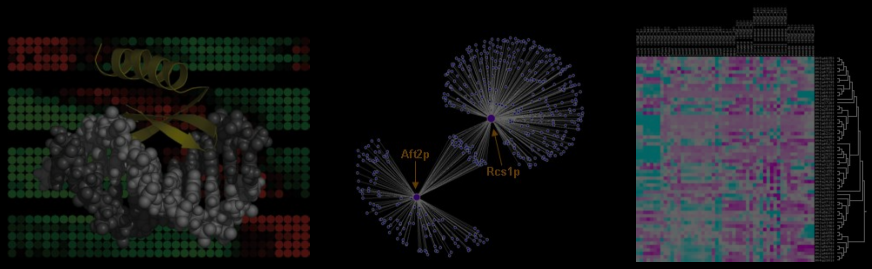
Genome research (2009)



PNAS (2008)

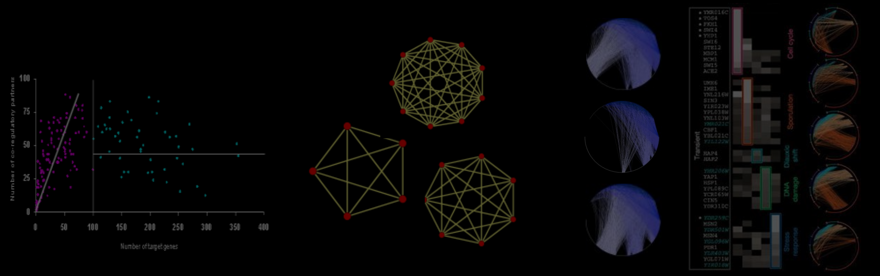
Molecules & regulation

- Predicting function of novel regulatory proteins



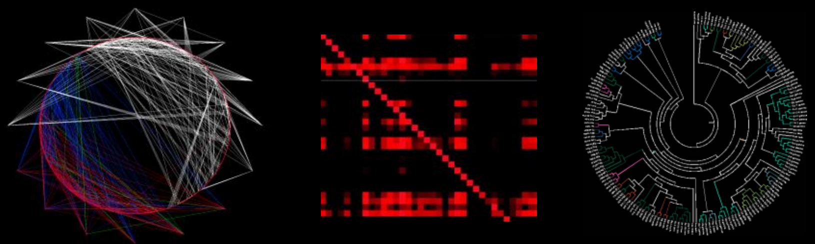
Systems & regulation

- Principles of regulation in cellular systems



Genomes & regulation

- Transcriptional regulation and genome organization



Outline

- Structure of the transcriptional regulatory network
- Evolution of the transcriptional regulatory network
- Dynamic nature of the transcriptional regulatory network
- Hierarchy and node-dynamics of regulatory networks

Detailed Outline

- Structure of the transcriptional regulatory network
  - Local network structure: network motifs
  - Global network structure: scale-free structure
- Evolution of the transcriptional regulatory network
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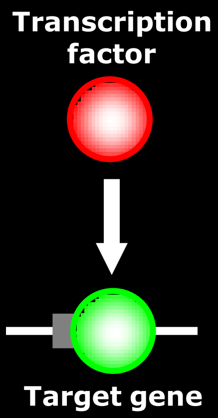
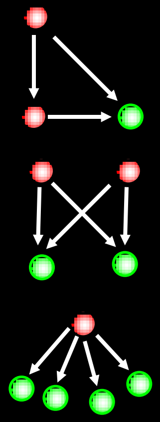
Organization of the transcriptional regulatory network analogy to the organization of the protein structures

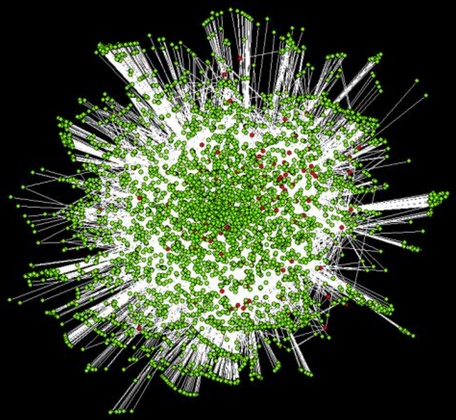
Basic unit
Transcriptional interaction



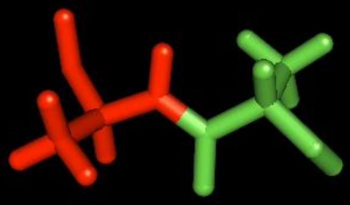
Local structure - Motifs
Patterns of interconnections



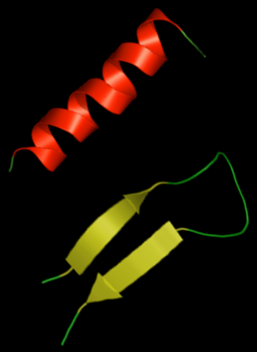
Global structure - Scale free network
All interactions in a cell



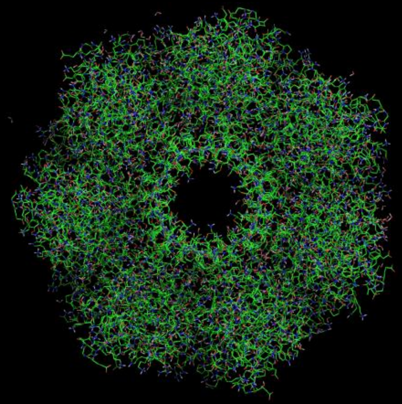
Basic unit
Peptide link between amino acids



Local structure
Secondary structure

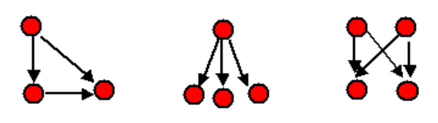


Global structure
Class/Fold

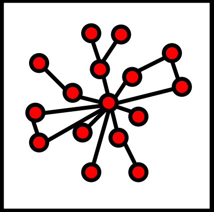


Properties of transcriptional networks

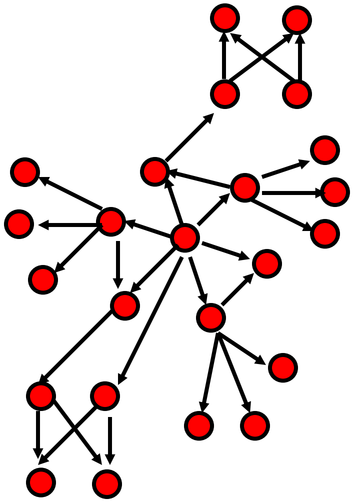
Local level: Transcriptional networks are made up of motifs which perform information processing task



Global level: Transcriptional networks are scale-free conferring robustness to the system



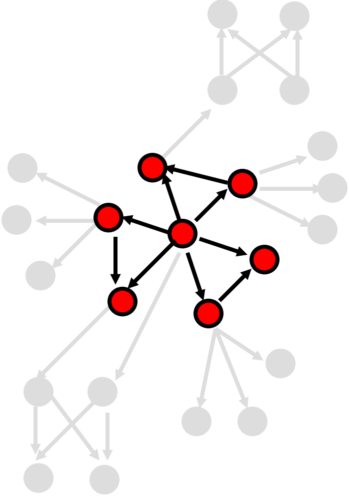
Transcriptional networks are made up of motifs



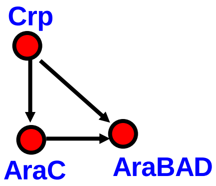
Network Motif

"Patterns of interconnections that recur at different parts and with specific information processing task"

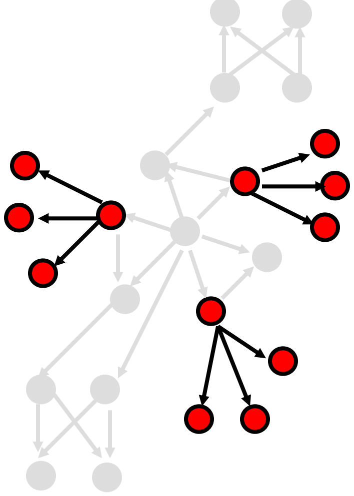
Function



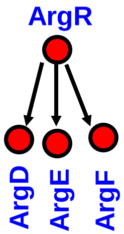
Feed Forward Motif



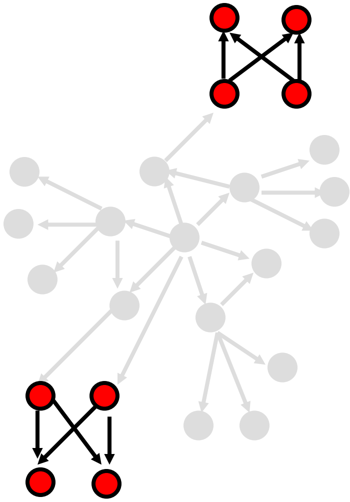
- **Responds to persistent signal**
- **Filters noise**



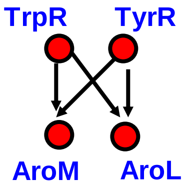
Single input Motif



- **Co-ordinates expression**
- **Enforces order in expression**
- **Quicker response**

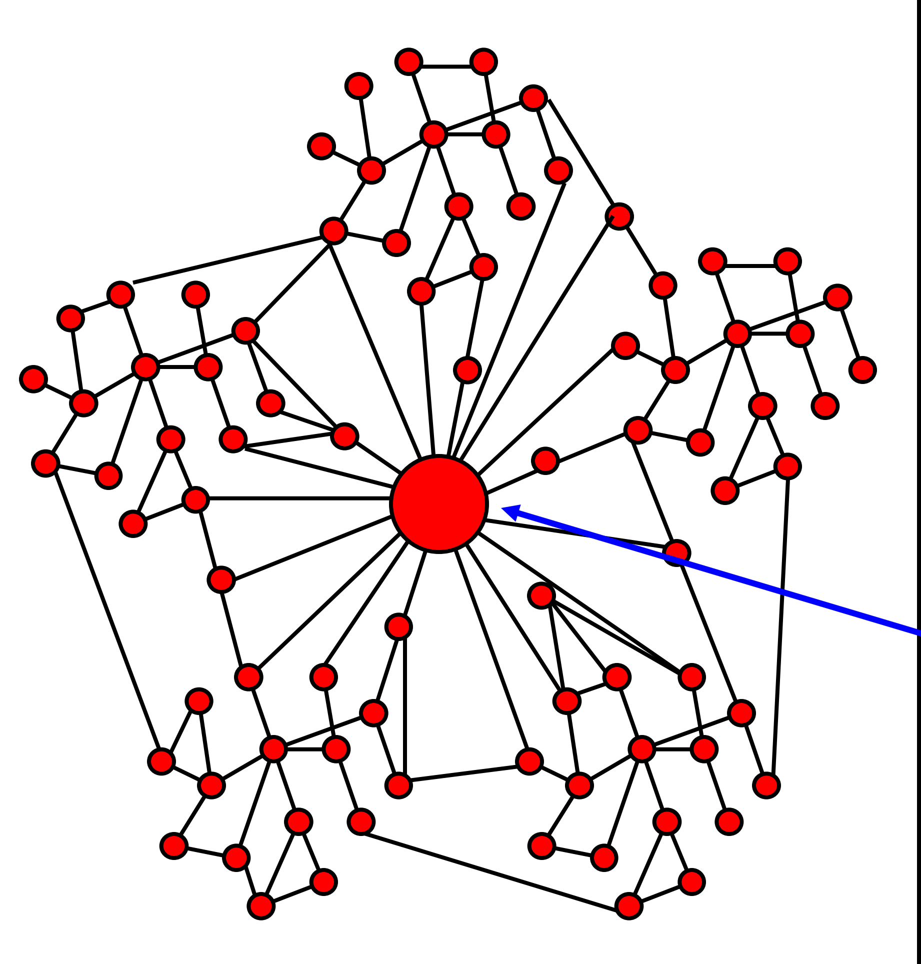


Multiple input Motif



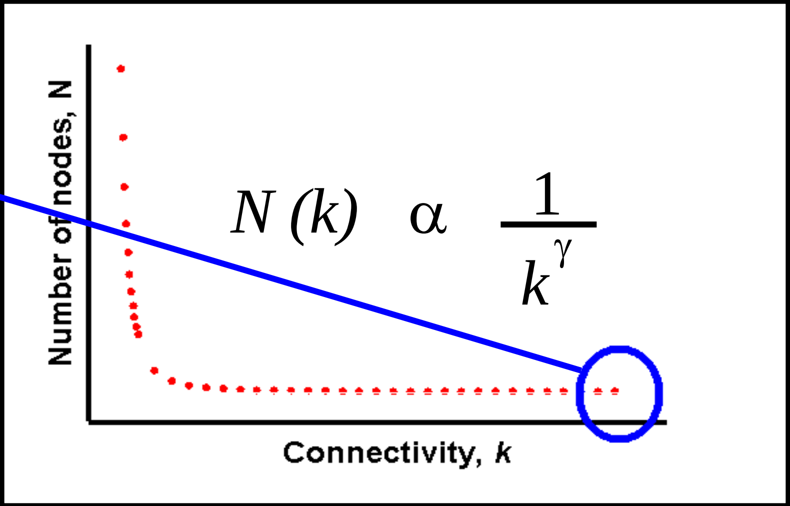
- **Integrates different signals**
- **Quicker response**

Transcriptional networks are scale-free



Scale-free structure

Presence of few
nodes with many links and many
nodes with few links



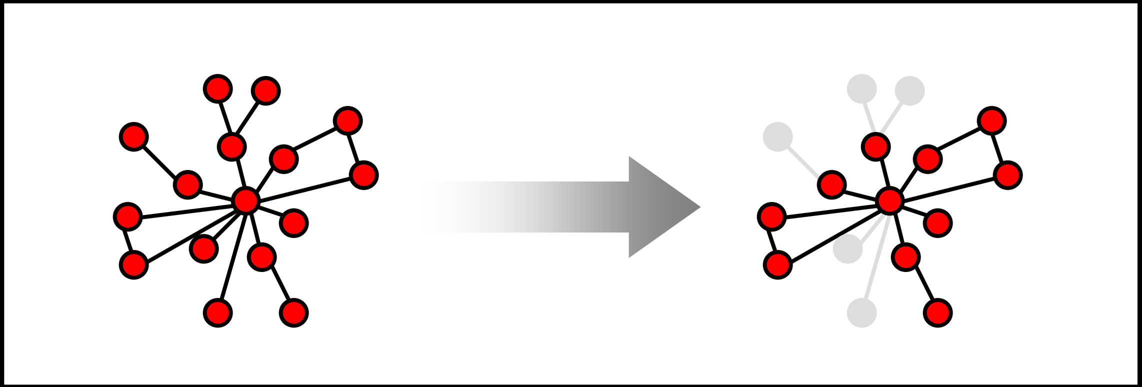
$$N(k) \;\alpha\; \frac{1}{k^{\gamma}}$$

Scale free structure provides robustness to the system

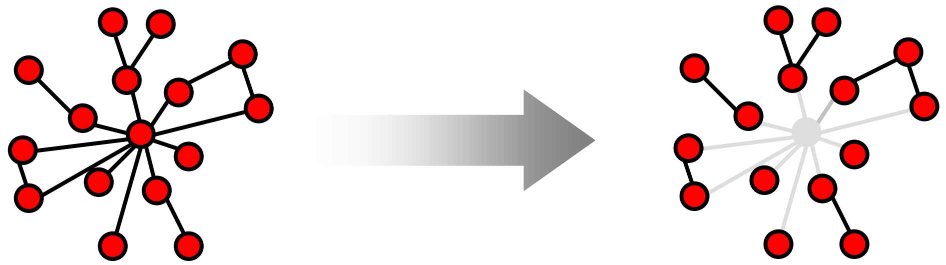
Scale-free networks exhibit robustness

Robustness – The ability of complex systems to maintain their function even when the structure of the system changes significantly

Tolerant to random removal of nodes (mutations)



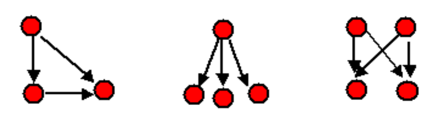
Vulnerable to targeted attack of hubs (mutations) – Drug targets



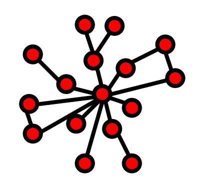
Hubs are crucial components in such networks

Summary I – Structure of transcriptional networks

Transcriptional networks are made up of motifs that have specific information processing task



Transcriptional networks have a scale-free structure which confers robustnessto such systems, with hubs assuming importance



Madan Babu M, Luscombe N et. al
Current Opinion in Structural Biology

Detailed Outline

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  - Local network structure: network motifs
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  - Evolution of local network structure
  - Evolution of global network structure
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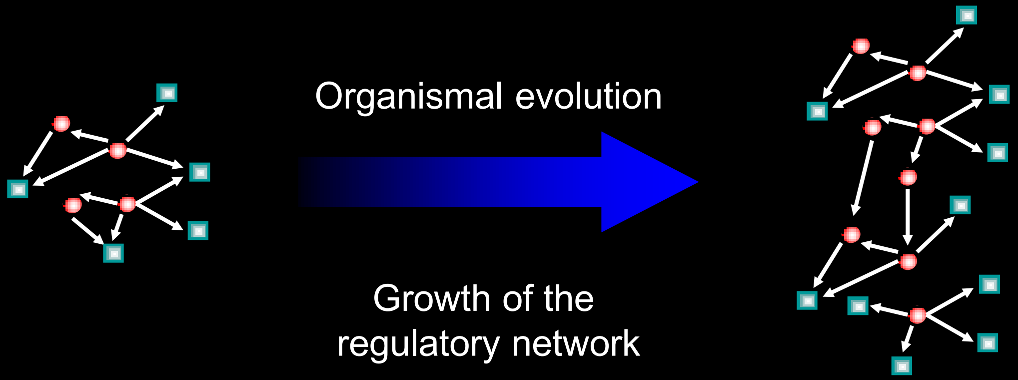
Evolution of the transcriptional regulatory network in yeast

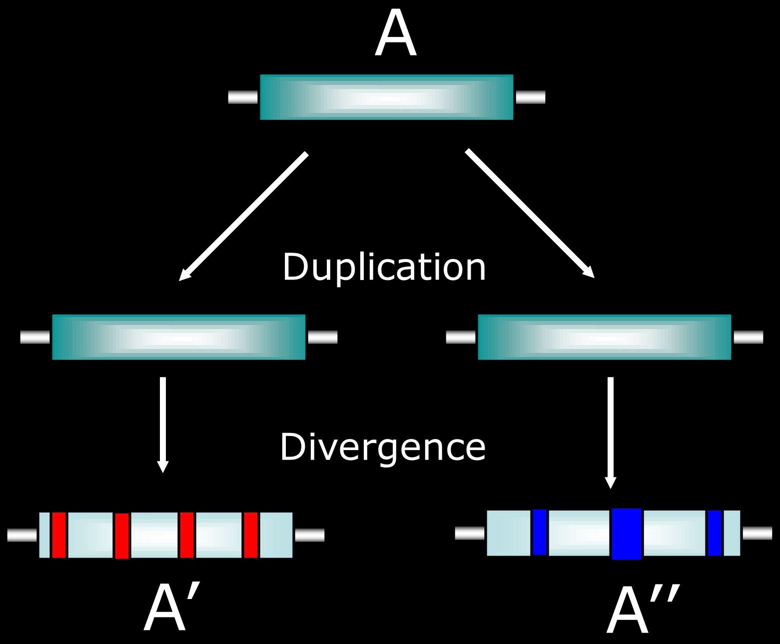


Organismal evolution

Growth of the regulatory network

How did the regulatory network evolve?

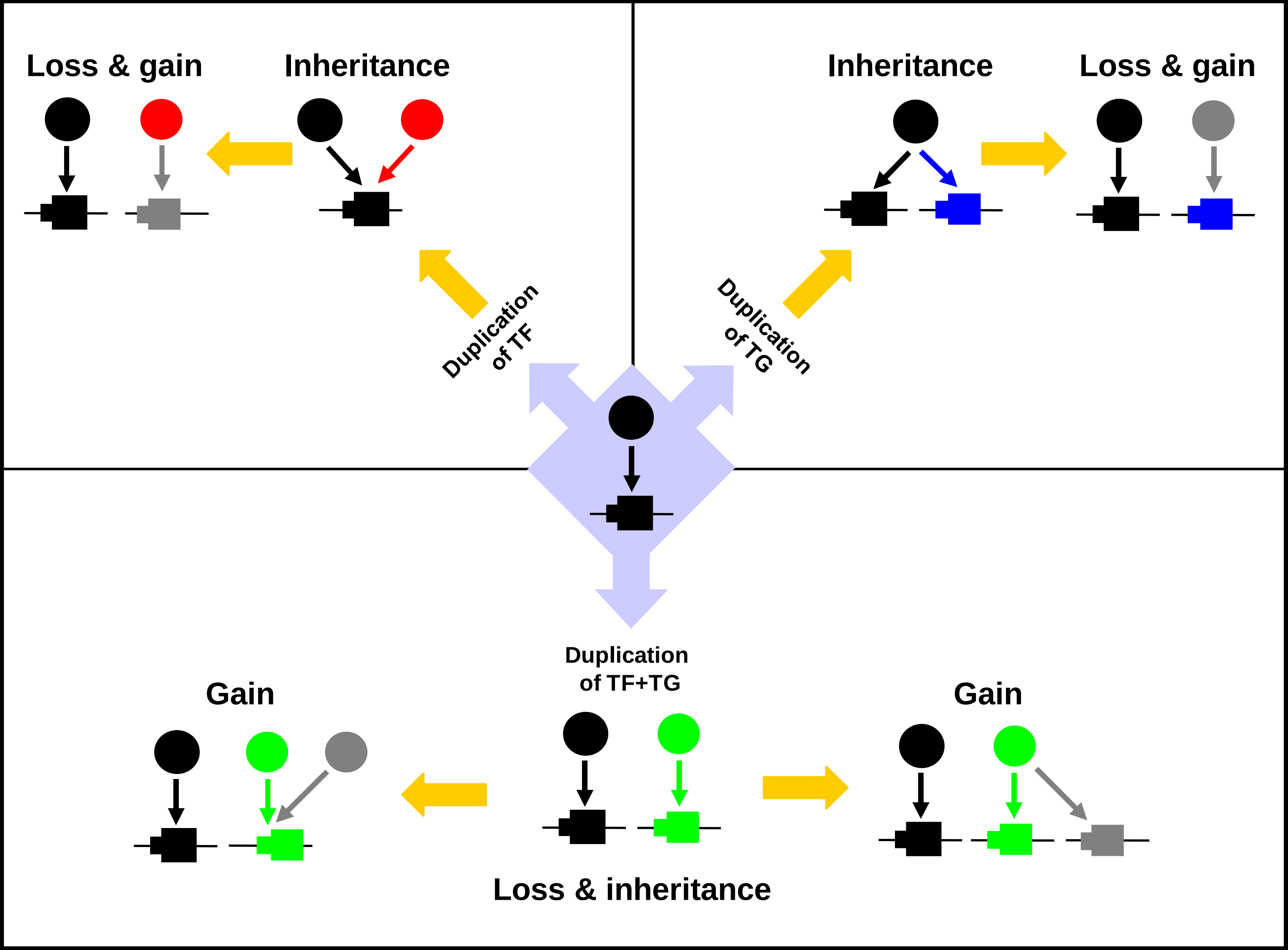
Mechanisms for the creation of new genes



Duplication & Divergence

OR Recombination/Innovation

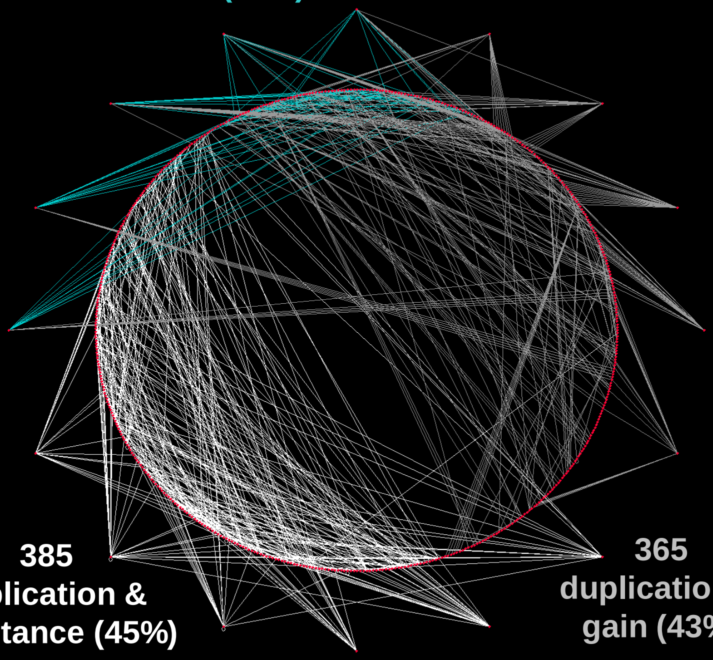
Gene duplication and network growth



Inheritance, loss and gain of interaction

Evolution of the transcriptional regulatory network

Entire network

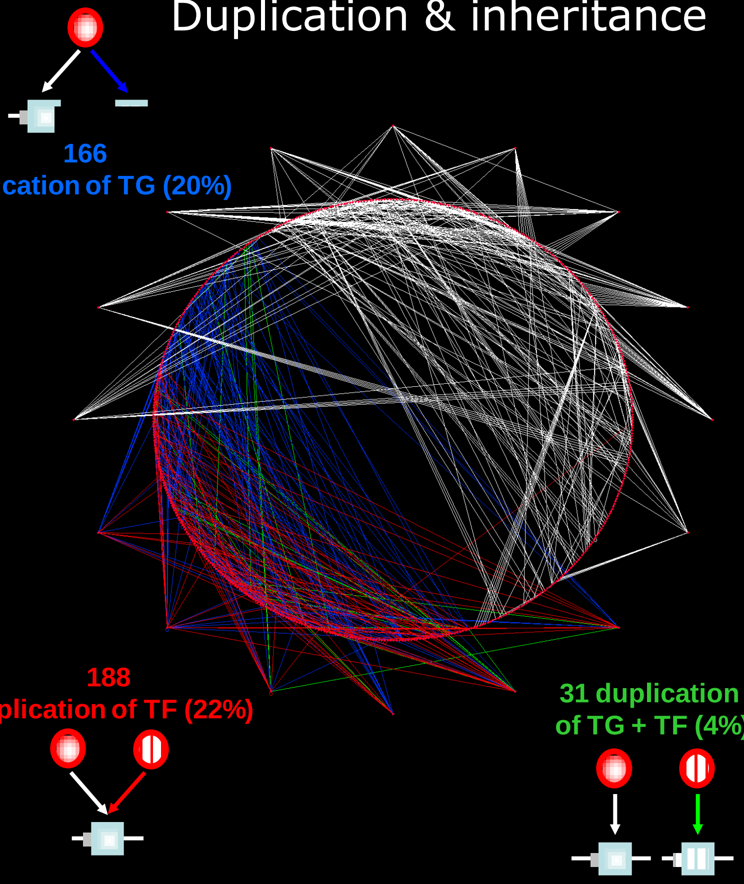


101
innovation (12%)

385
duplication &
inheritance (45%)

365
duplication &
gain (43%)

Duplication & inheritance



166
duplication of TG (20%)

188
duplication of TF (22%)

31 duplication
of TG + TF (4%)

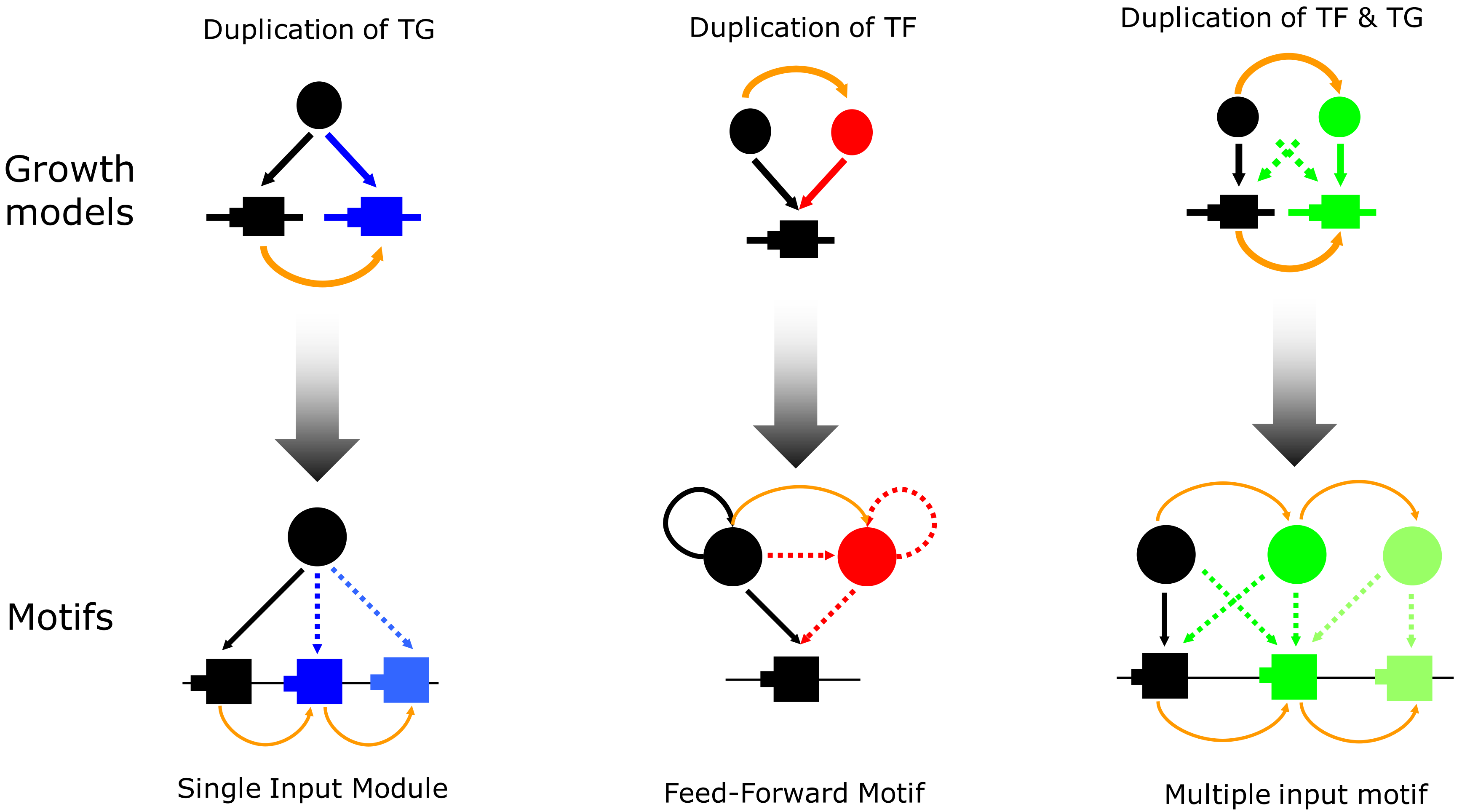
~90% of the network has evolved by duplication followed by Inheritance, loss and gain of interaction

How can such events affect local and global network structure?

Are the motifs and scale free structure products of duplication events ?

Are the motifs and scale free structure selected for in evolution ?

Duplication models and evolution of network motifs



Duplication of TG

Duplication of TF

Duplication of TF & TG

Growth models

Motifs

Single Input Module

Feed-Forward Motif

Multiple input motif

Evolution of local network structure

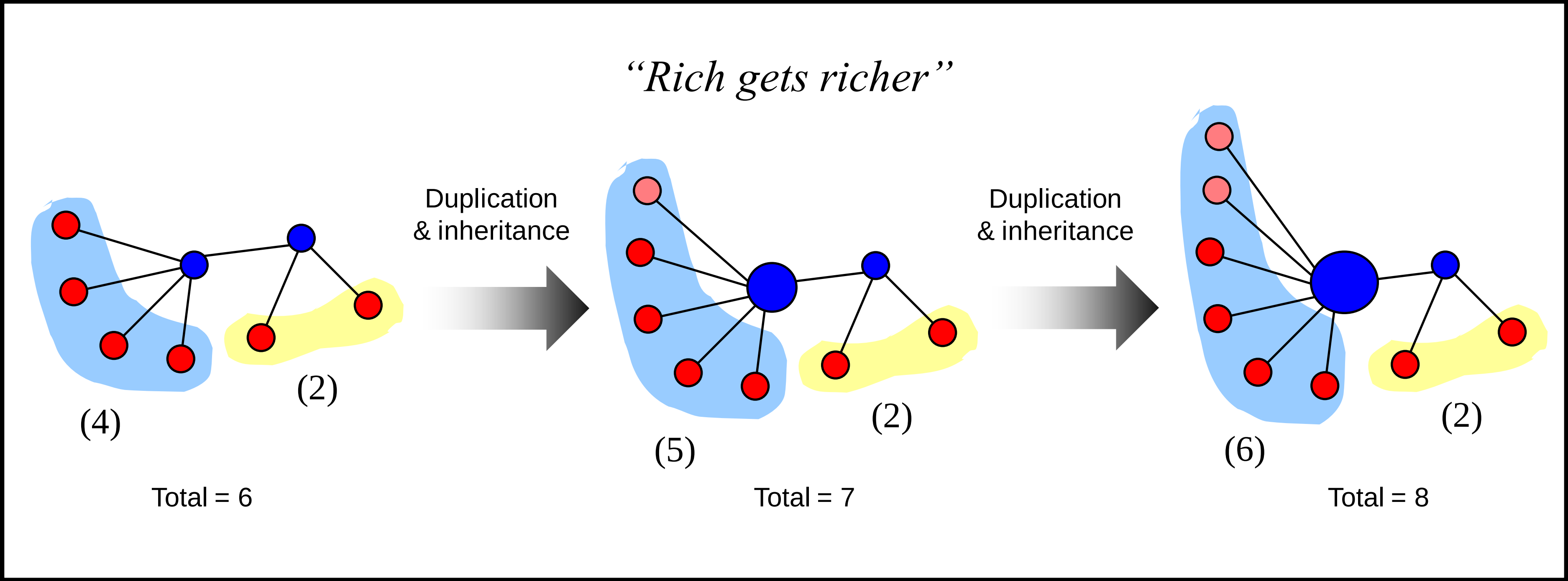
Very rarely we find instances where duplication events have resulted in the formation of network motifs

↓

Network motifs have evolved independently (convergent evolution) multiple times because they confer specific properties to the network

Duplication and evolution of scale free structure

Growth by gene duplication and inheritance of interaction can explain evolution of scale-free structure



Regulatory hubs should control more duplicate genes

Evolution of global network structure

Regulatory hubs do not regulate duplicate genes more often than any other normal transcription factor

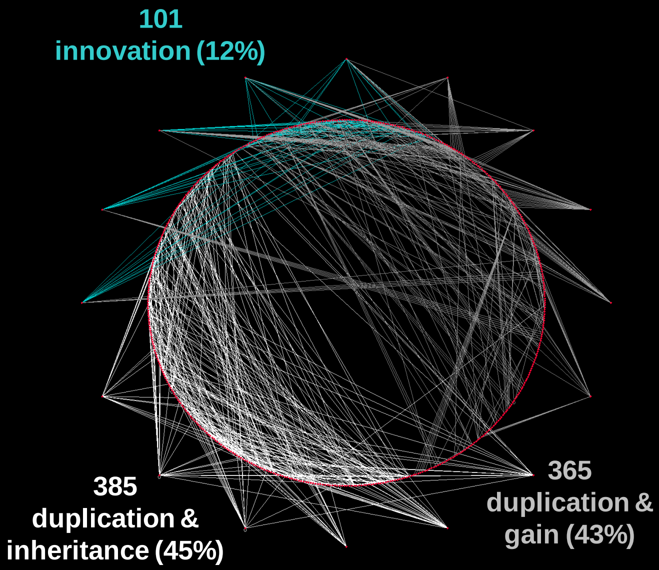
↓

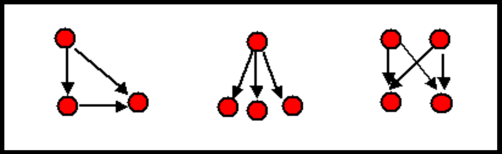
Scale free structure has been selected for in evolution and is not a product of duplication events

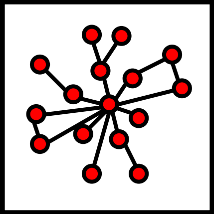
Summary II - Evolution

Gene duplication followed by inheritance of interaction and gain of new interactions have contributed to 90% of the network

Network motifs and the scale free structure are not products of duplication events, but have been selected for in evolution







Teichmann SA & Madan Babu M
Nature Genetics

Detailed Outline

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  - Local network structure: network motifs
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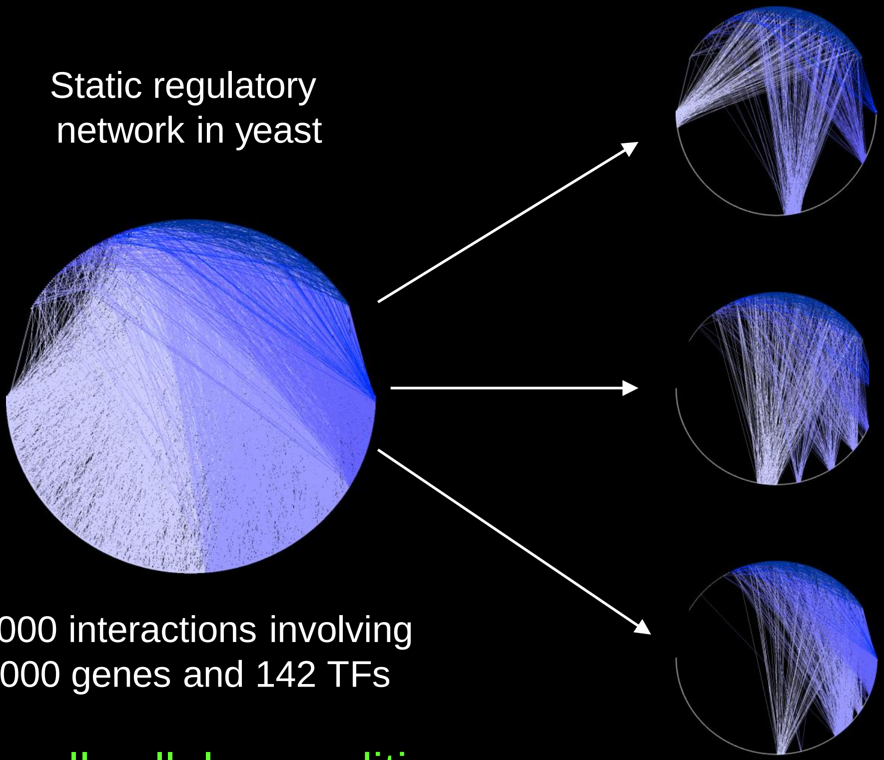
- Hierarchy and node-dynamics of regulatory networks

Temporal dynamics of the regulatory networks



Static regulatory network in yeast

~ 7000 interactions involving 3000 genes and 142 TFs

Across all cellular conditions

Cell cycle

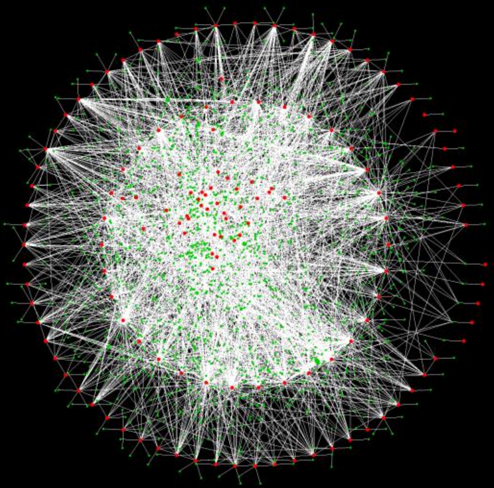
Sporulation

Stress

How does the local structure change in different cellular conditions?

How does the global structure change in different conditions?

Integrating gene regulatory network with expression data

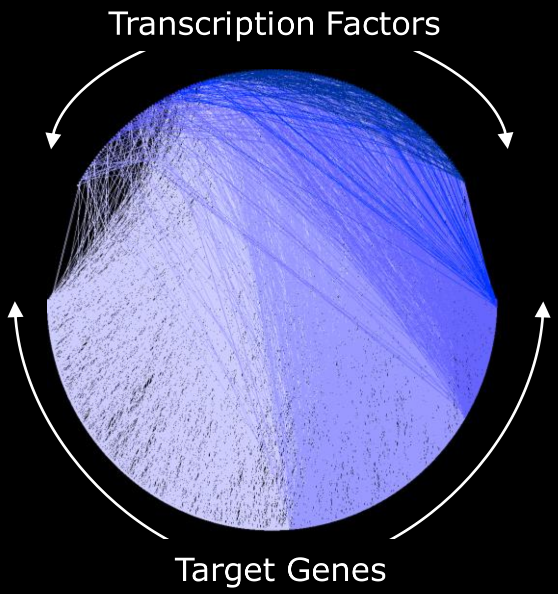


142 TFs
3,820 TGs
7,074 Interactions

Gene expression data

for 5 cellular conditions

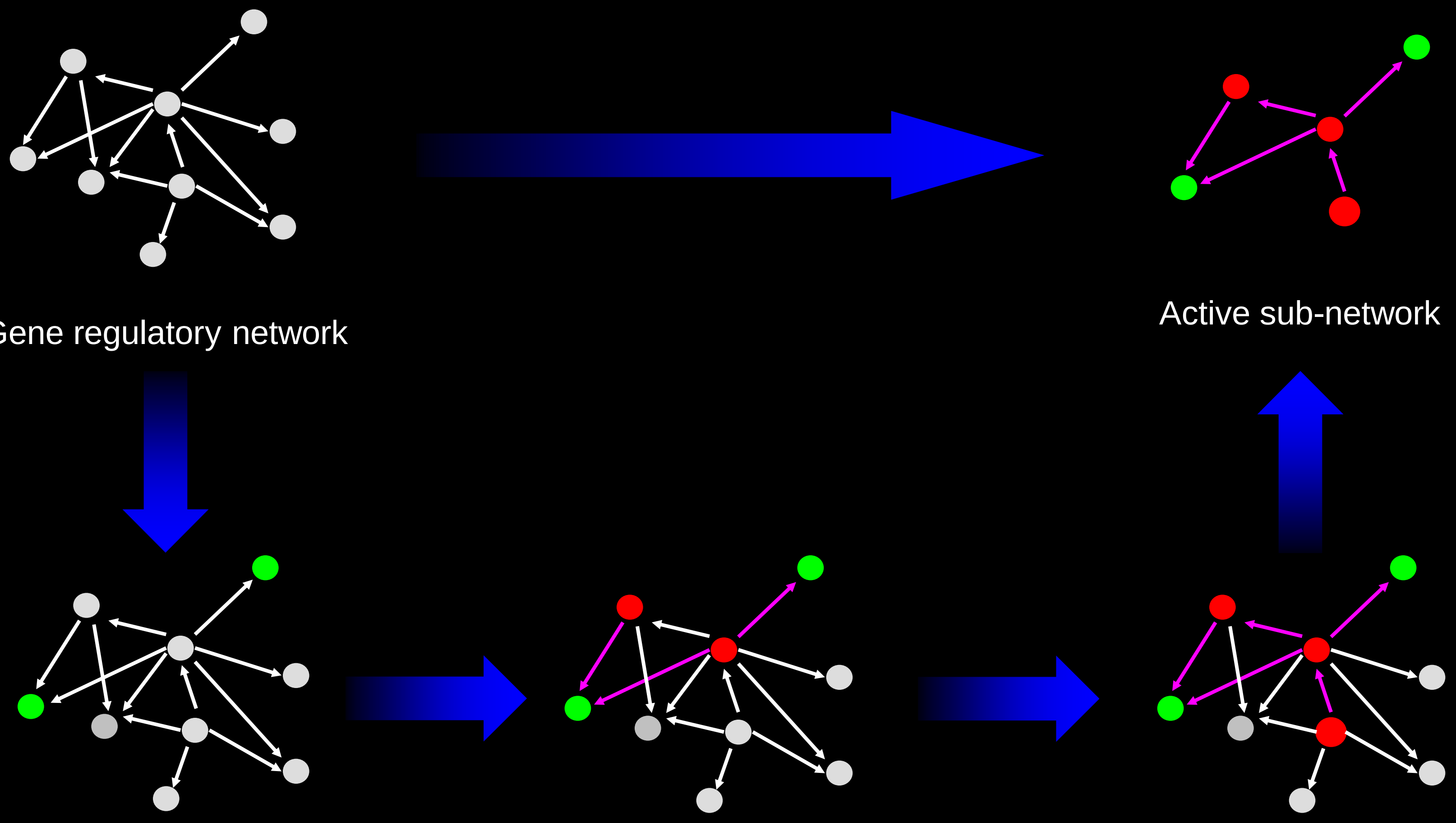
Cell-cycle
Sporulation
DNA damage
Diauxic shift
Stress



142 TFs
1,808 TGs
4,066 Interactions

- ■ **1 condition**
- ■ **2 conditions**
- ■ **3 conditions**
- ■ **4 conditions**
- ■ **5 conditions**

Back-tracking method to find active sub-networks



Gene regulatory network

Active sub-network

Identify differentially regulated genes

Find TFs that regulate the genes

Find TFs that regulate these TFs

Regulatory program specific transcriptional networks

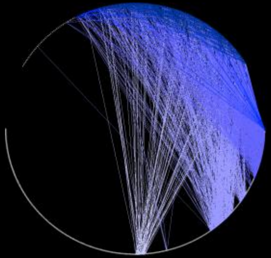
Binary Processes

Regulatory programs involved in survival

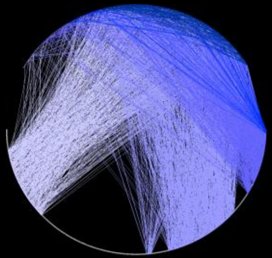
Multi-step Processes

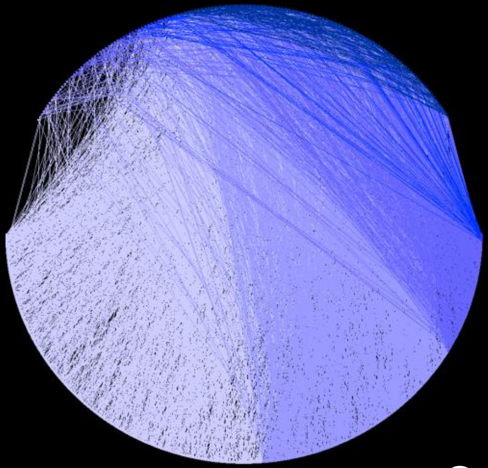
Regulatory programs involved in development

Stress

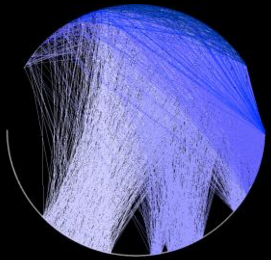


Diauxic shift

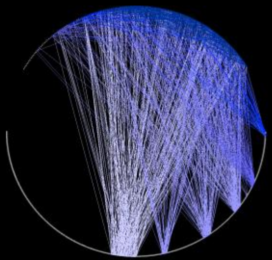




DNA damage



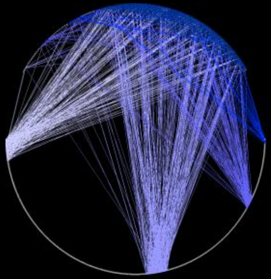
Sporulation



Pre-sporulation → Sporulation → Germination

E L M

Cell cycle

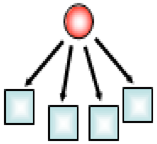
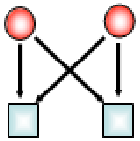
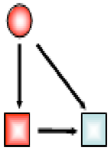


G0 → G1 → S → G2 → M

Temporal dynamics of local structure

Multi-step regulatory programs (development)

Binary regulatory programs (survival)

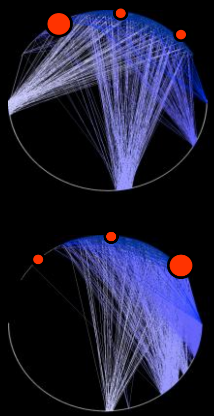
	Motifs		**Cell cycle**	**Sporulation**	**Diauxic shift**	**DNA damage**	**Stress response**
fast acting *&* *direct*	**SIM**		32.0%	38.9%	57.4%	55.7%	59.1%
fast acting *&* *direct*	**MIM**		23.7%	16.6%	23.6%	27.3%	20.2%
slow acting *&* *indirect*	**FFL**		44.3%	44.5%	19.0%	17.0%	20.7%

Network motifs that allow for efficient execution of regulatory steps are preferentially used in different regulatory programs

Temporal dynamics of global structure (hubs)

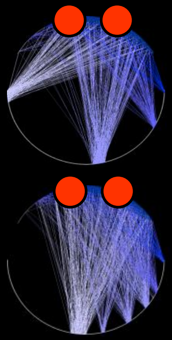
Condition specific hubs

Each regulatory program is triggered by specific hubs

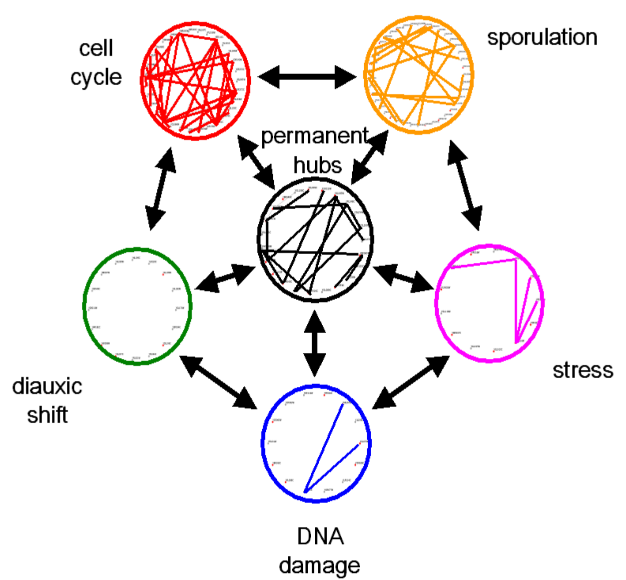


Permanent hubs

Active across all regulatory programs

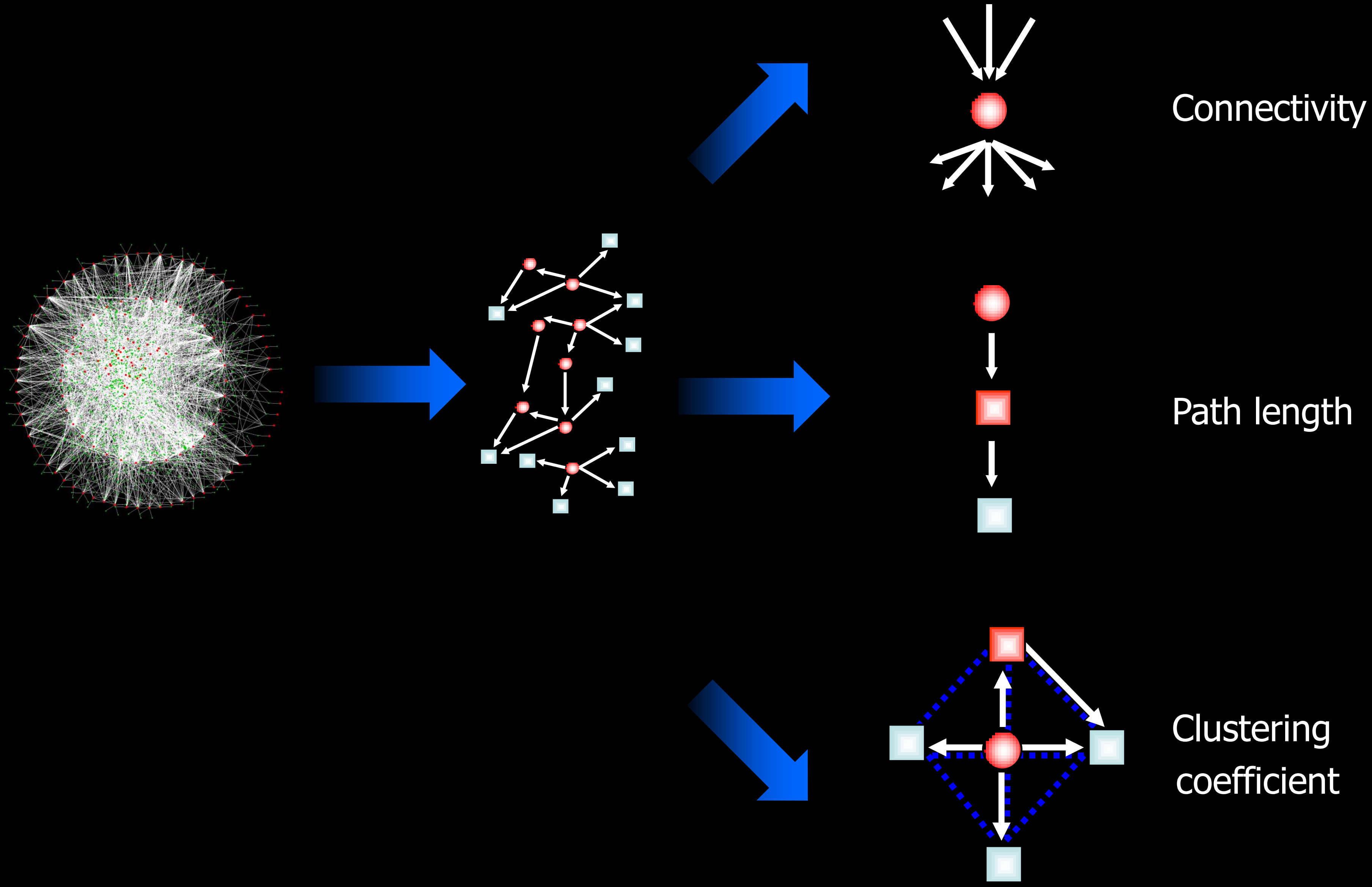


Hubs regulate other hubs to trigger cellular events



This suggests a dynamic structure which transfers 'power' between hubs to trigger distinct regulatory programs (developmental & survival)

Network Parameters

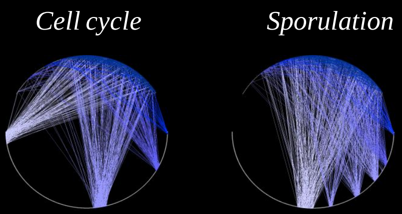


Connectivity

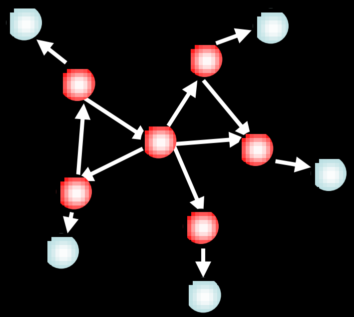
Path length

Clustering coefficient

multi-stage conditions



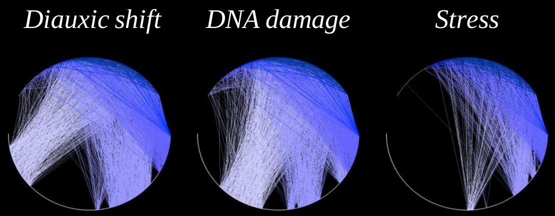
Cell cycle *Sporulation*



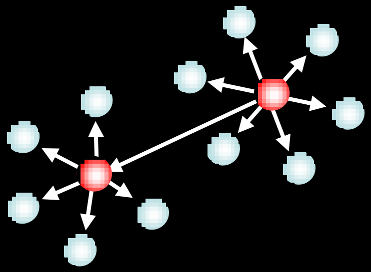
- fewer target genes per TF
- longer path lengths
- more inter-regulation between TFs

Fidelity in response

binary conditions



Diauxic shift *DNA damage* *Stress*



- more target genes per TF
- shorter path lengths
- less inter-regulation between TFs

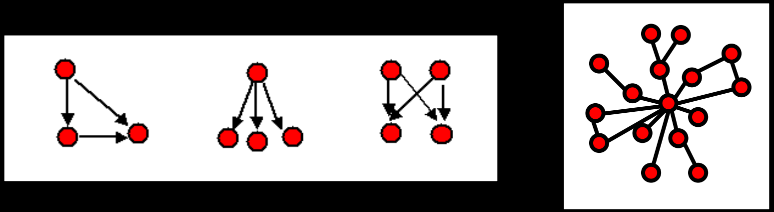
Quick response

Sub-networks re-wire both their local and global structure to respond to cellular conditions efficiently

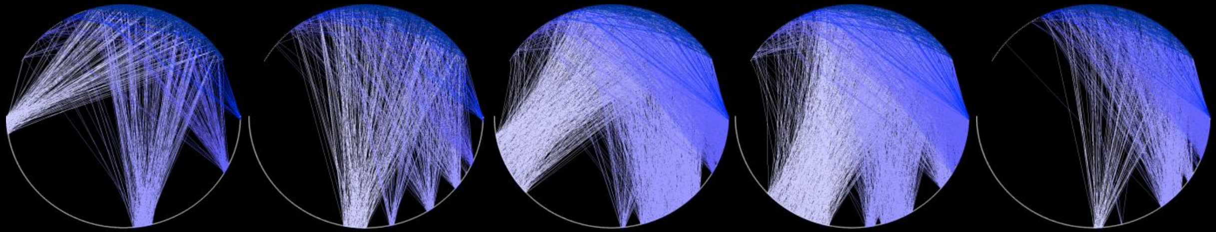
Luscombe N, Madan Babu M et. al
Nature (2004)

Summary III - Dynamics

Network motifs are preferentially used under the different cellular conditions and different proteins act as regulatory hubs in different cellular conditions



Sub-networks have evolved both their local structure and global structure to respond to cellular conditions efficiently



Luscombe N, Madan Babu M et. al
Nature (2004)

Detailed Outline

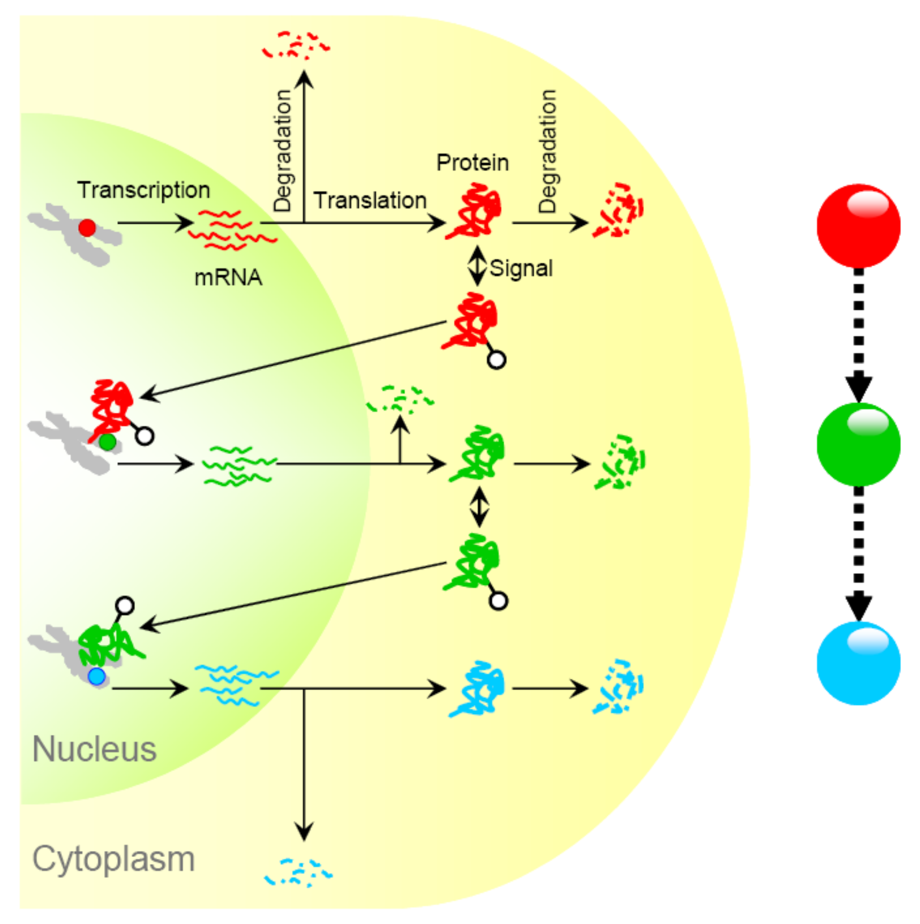
- Structure of the transcriptional regulatory network
  - Local network structure: network motifs
  - Global network structure: scale-free structure

- Evolution of the transcriptional regulatory network
  - Evolution of local network structure
  - Evolution of global network structure

- Dynamic nature of the transcriptional regulatory network
  - Dynamics of local network structure
  - Dynamics of global network structure

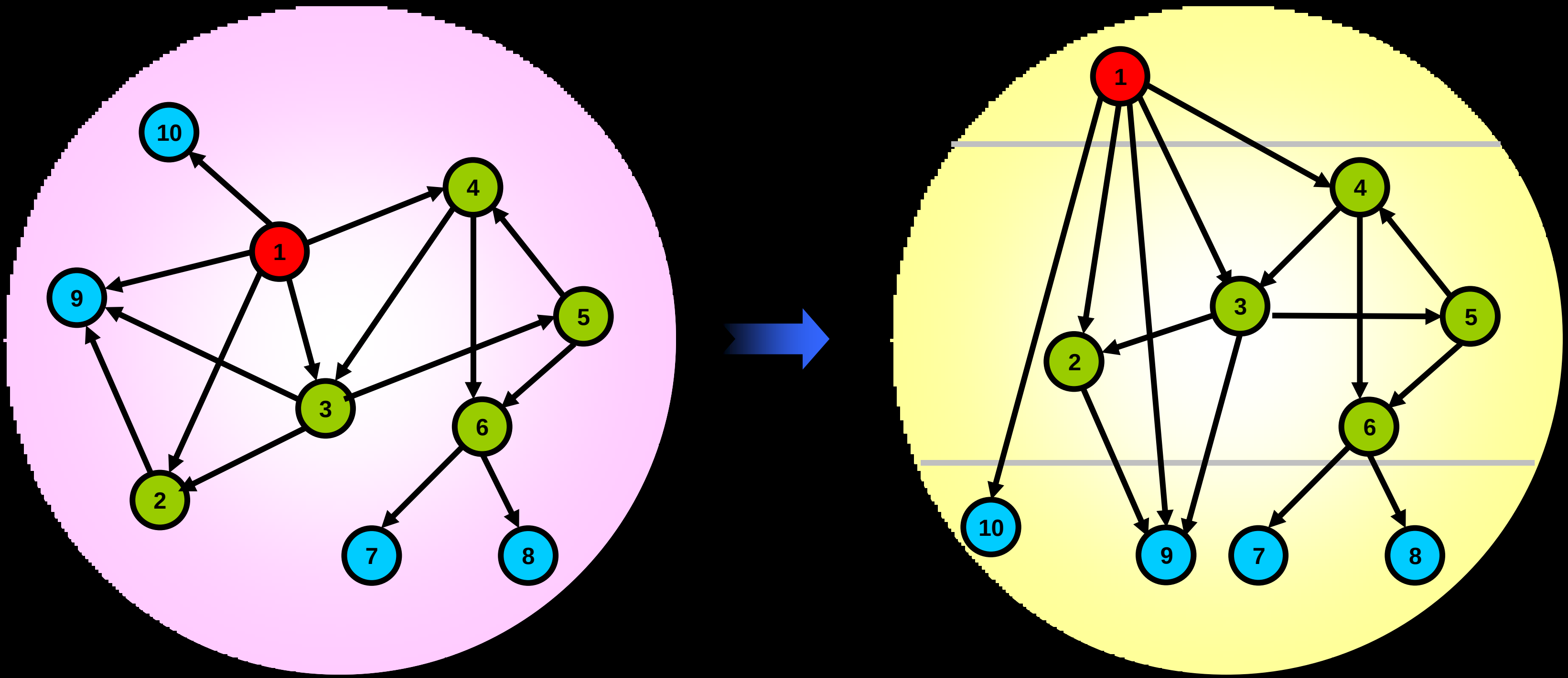
- Hierarchy and node-dynamics of regulatory networks
  - Hierarchy in transcriptional networks
  - Noise in gene expression – implications for transcriptional networks

Dynamics in transcription and translation



Each node in the network represents several entities (gene, mRNA, and protein) and events (transcription, translation, degradation, etc) that are compressed in both space and time

Higher-order organization of transcriptional regulatory networks



Inherent higher-order organization

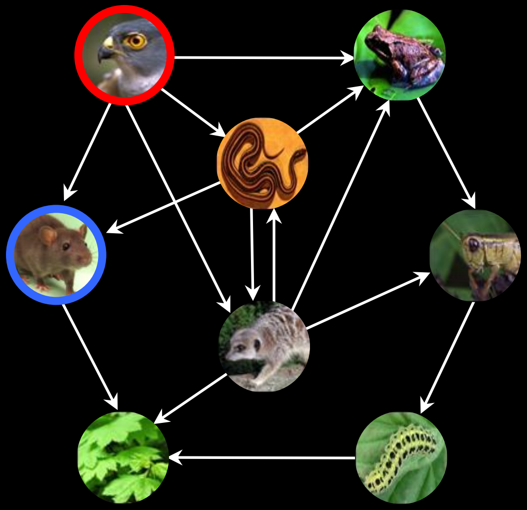
Flat structure

Hierarchical structure

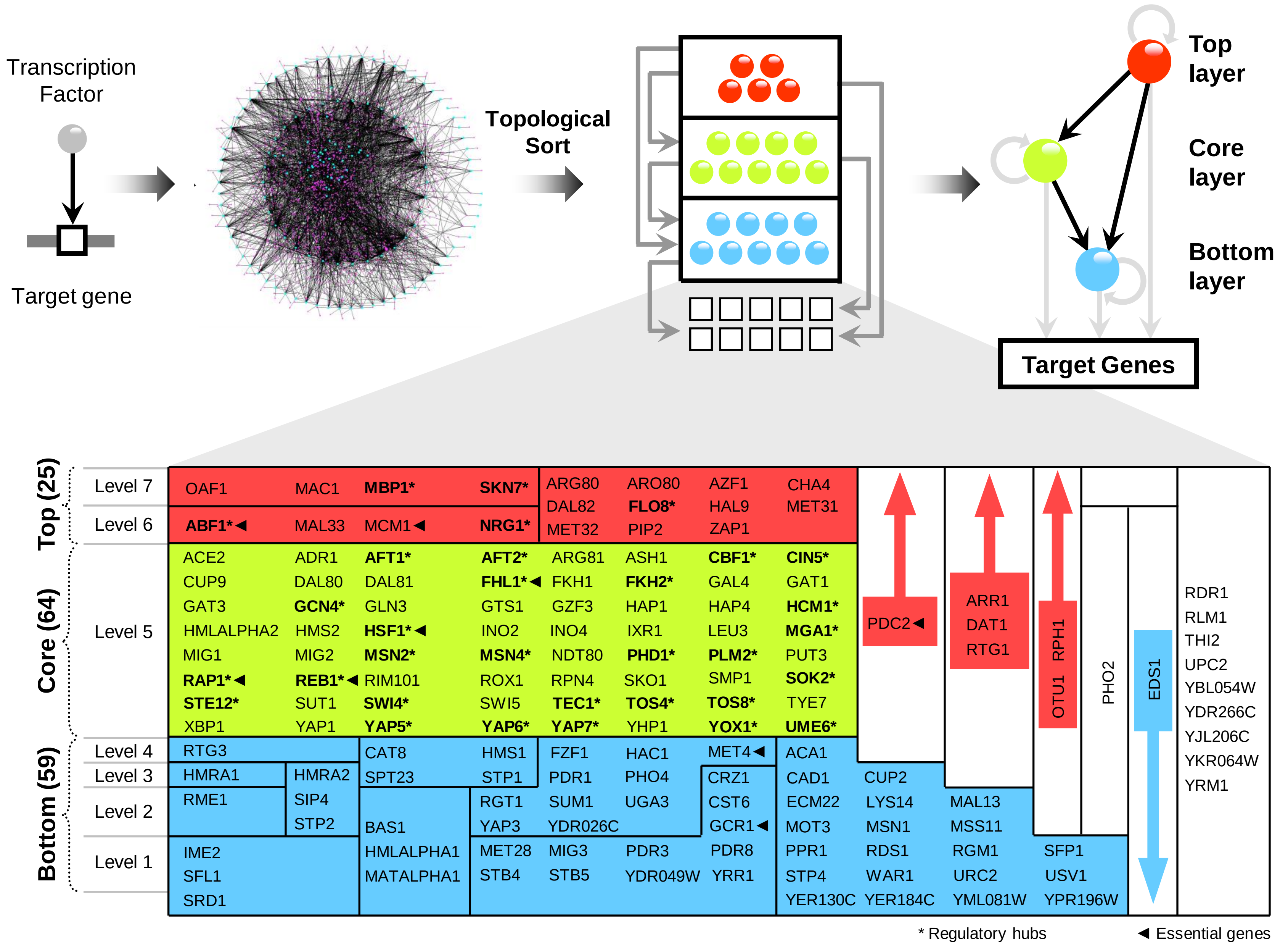
Methods to infer hierarchical organization in networks

Food web network

predator ⟶ prey

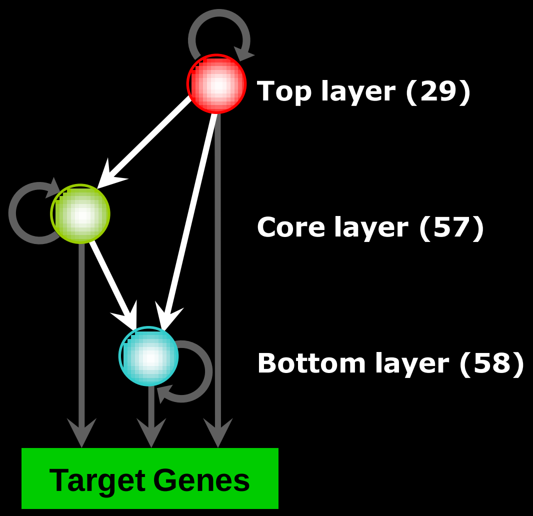


Hierarchical organization of the yeast regulatory network



Hierarchical organization of regulatory proteins

Regulatory proteins are hierarchically organized into three basic layers

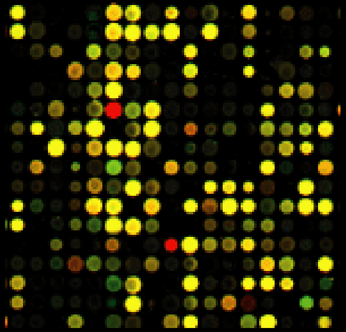


Do TFs in the different hierarchical levels have distinct dynamic properties?

Datasets characterizing dynamics of transcription and translation

Transcript abundance

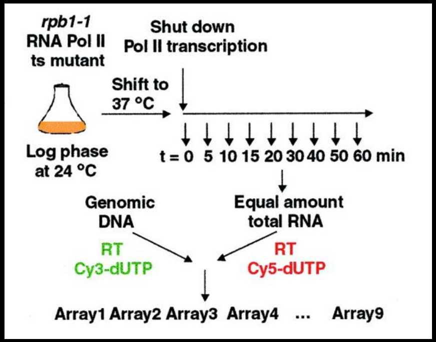
(Holstege et al., 1998)



Transcript abundances for yeast grown in YPD (S. cerevisiae) and Edinburgh minimal medium (S. pombe) were determined by using an Affymetrix high density oligonucleotide array.

Transcript half-life

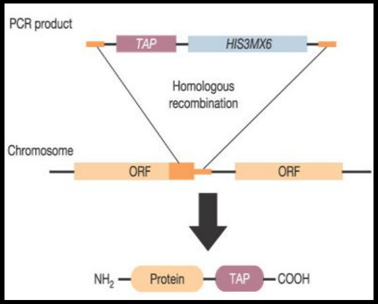
(Wang et al., 2002)
(Yang et al., 2003)



Transcript half-lives were determined by obtaining transcript levels over several minutes after inhibiting transcription. This was done using the temperature sensitive RNA polymerase rpb1-1 mutant S. cerevisiae strain.

Protein abundance

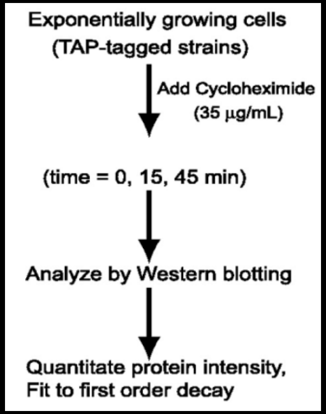
(Ghaemmaghami et al, 2003)



Estimates of the endogenous protein expression levels during log-phase were obtained by TAP-tagging every yeast protein for S. cerevisiae.

Protein half-life

(Belle et al, 2006)

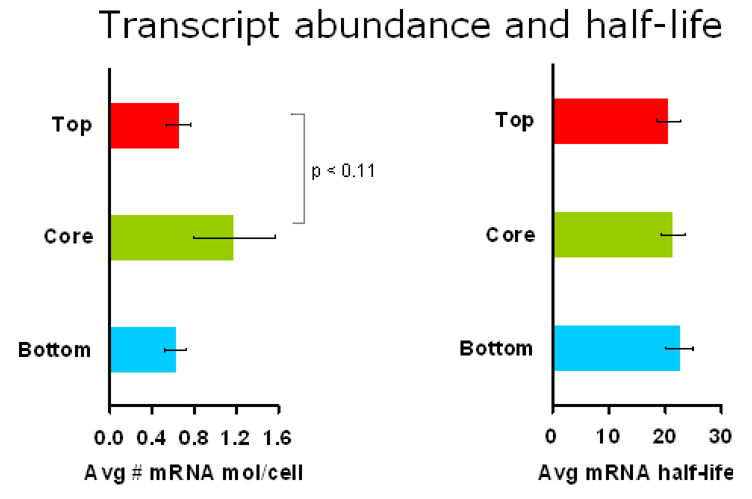


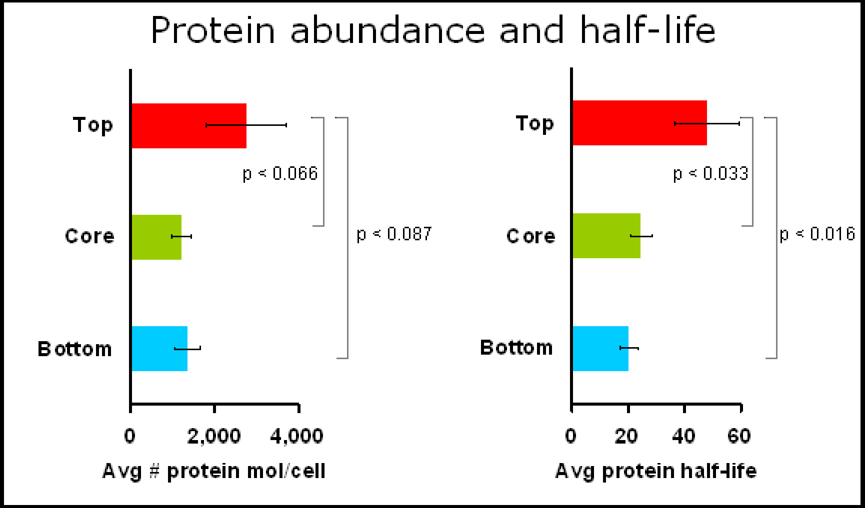
Protein half-lives were determined by first inhibiting protein synthesis via the addition of cyclohexamide and by monitoring the abundance of each TAP-tagged protein in the yeast genome as a function of time.

Transcriptional dynamics

Translational dynamics

Regulation of regulatory proteins within the hierarchical framework


Transcript abundance and half-life


Protein abundance and half-life

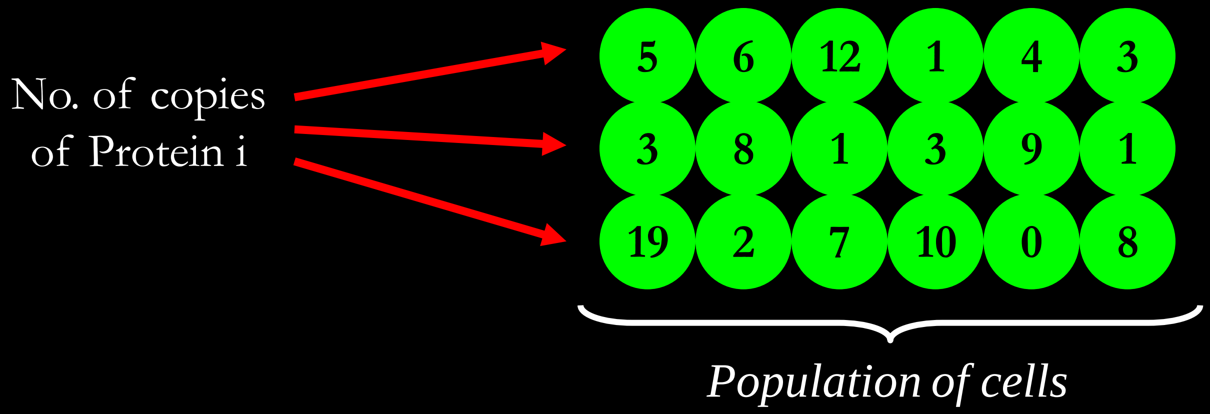
Regulation of transcript abundance or degradation does not appear to be a major control mechanism by which the steady state levels of TFs are controlled

Regulation of protein abundance and degradation appears to be a major control mechanism by which the steady state levels of TFs are controlled

post-transcriptional regulation plays an important role in ensuring the availability of right amounts of each TF within the cell

Noise in protein levels in a population of cells

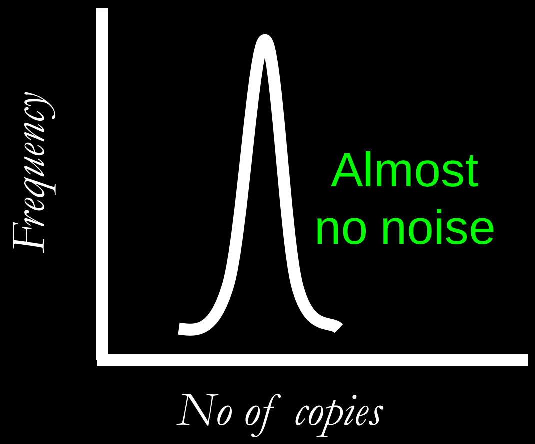
Noise in a population of cells can be beneficial where phenotypic diversity could be advantageous but detrimental if homogeneity and fidelity in cellular behaviour is required

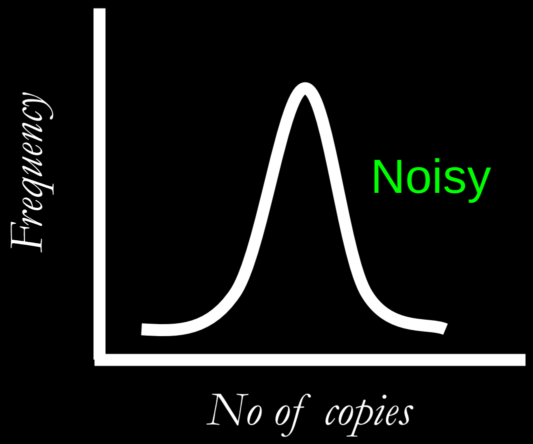


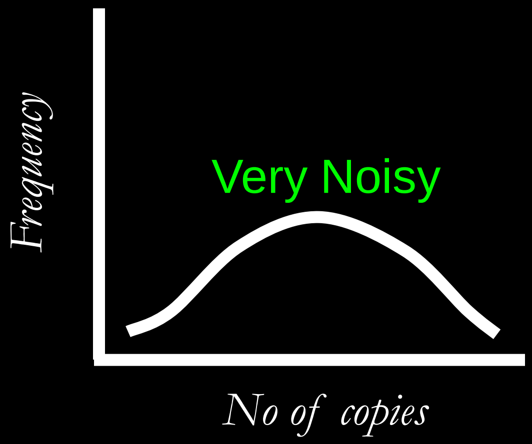
No. of copies
of Protein i

5	6	12	1	4	3
3	8	1	3	9	1
19	2	7	10	0	8

Population of cells

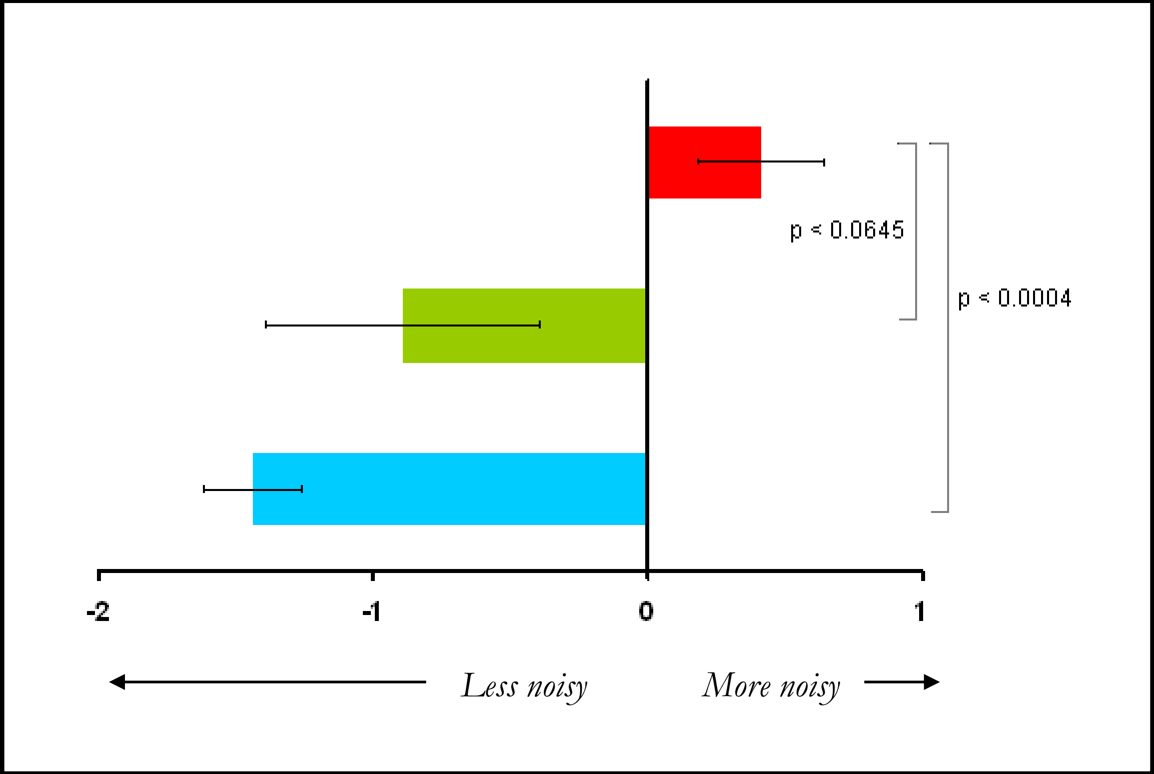


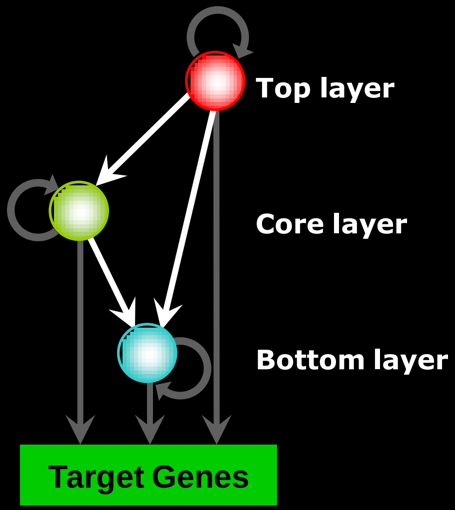




Lopez-Maury L, Marguaret S and Bahler J, **Nat Rev Genet 2008**

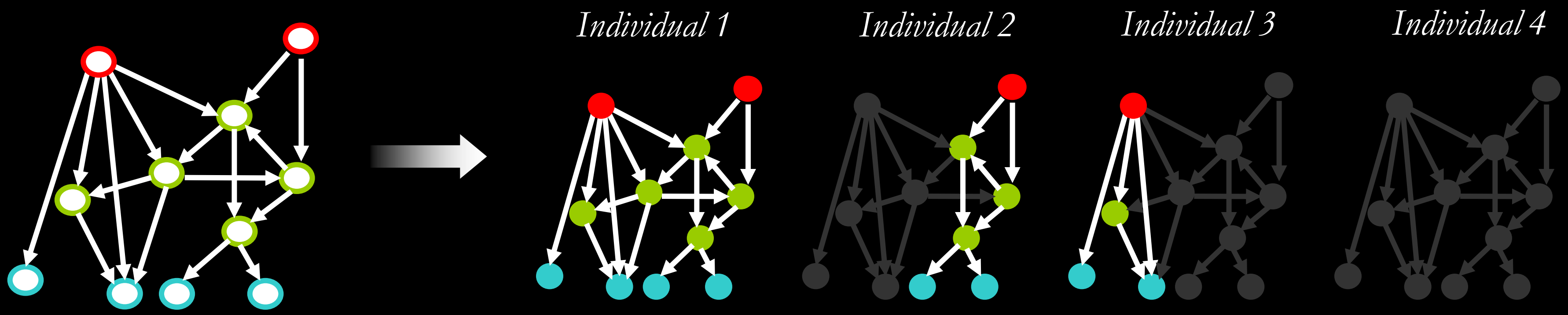
Noise levels of regulatory proteins





Regulatory proteins in the top layer are more noisy than the ones in the core or bottom layer

Implication

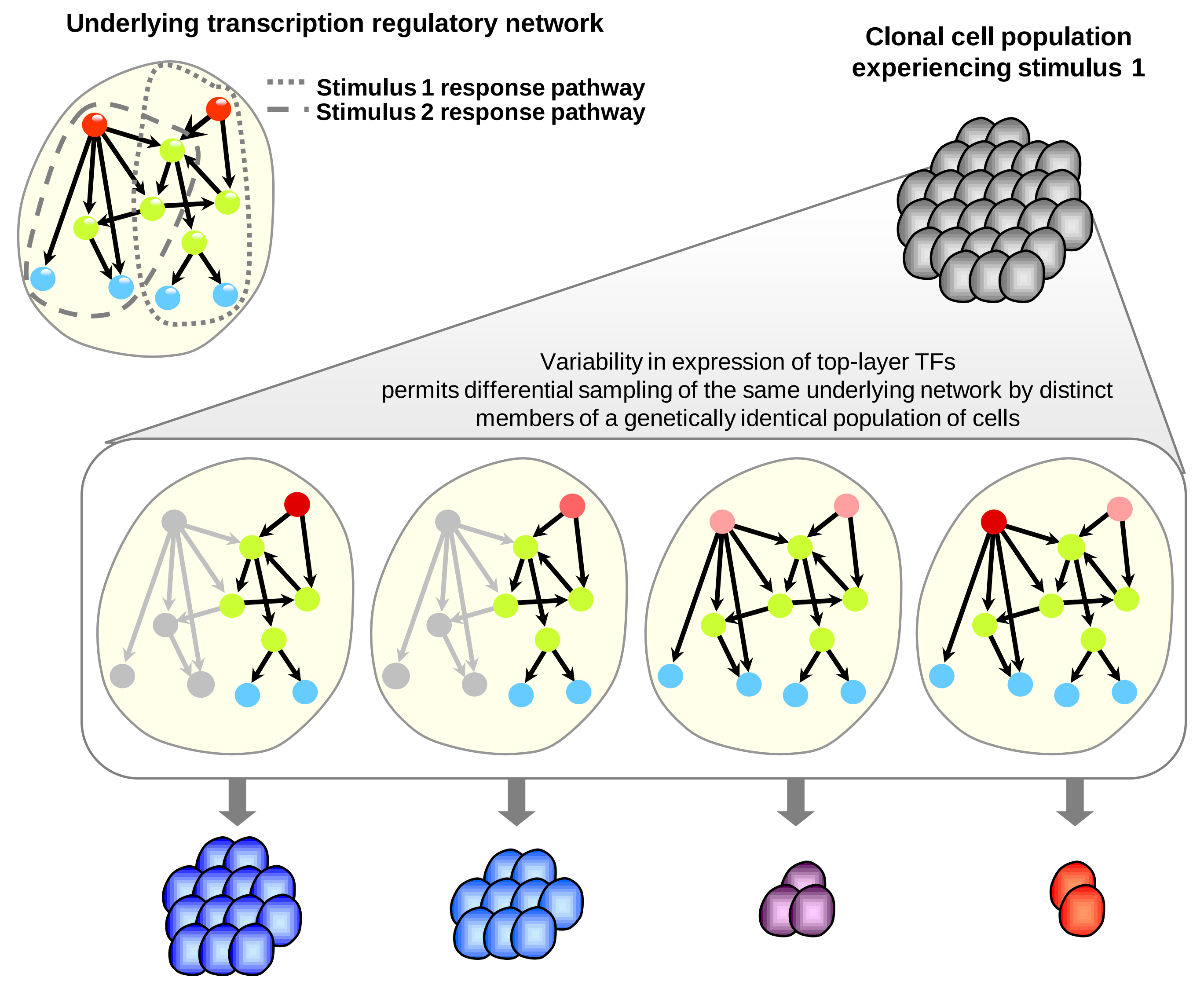


Underlying network

Differential utilization of the same underlying network by different individuals in a population of cells

Noise in TF expression may permit differential utilization of the same underlying regulatory network in different individuals of a population

Non-genetic variation due to differential transcription network utilization



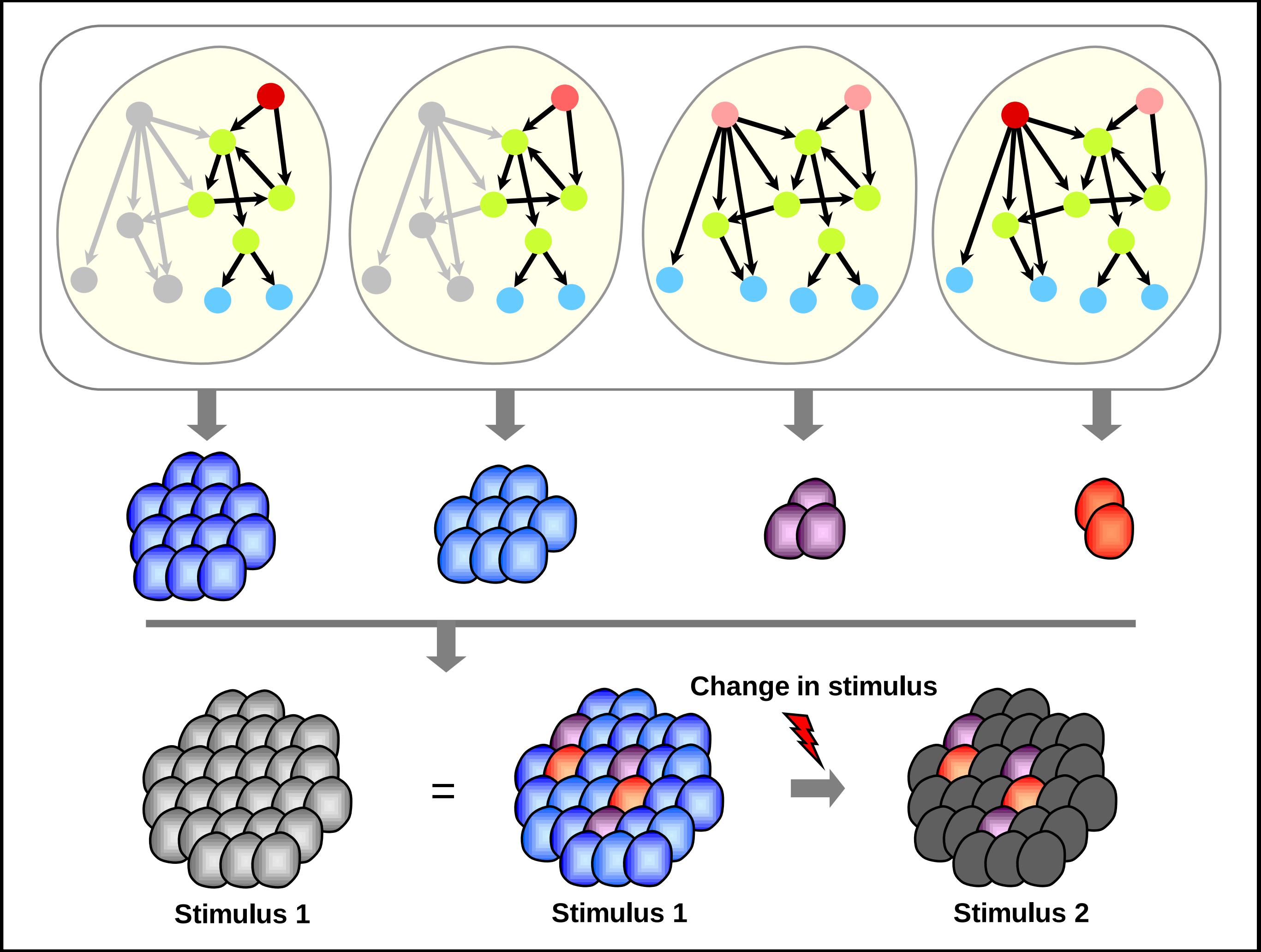
Underlying transcription regulatory network

⋯ Stimulus 1 response pathway
– – Stimulus 2 response pathway

Clonal cell population experiencing stimulus 1

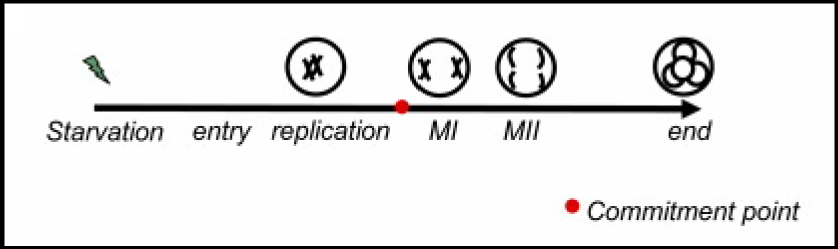
Variability in expression of top-layer TFs
permits differential sampling of the same underlying network by distinct
members of a genetically identical population of cells

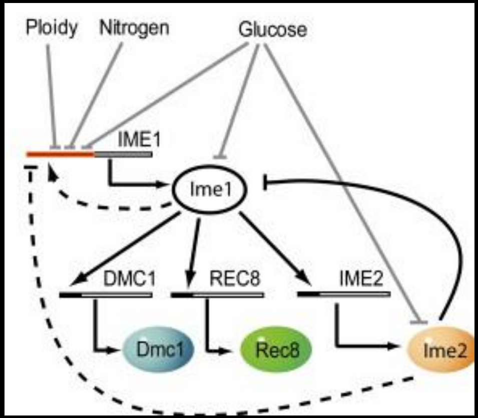
Non-genetic variation due to differential transcription network utilization



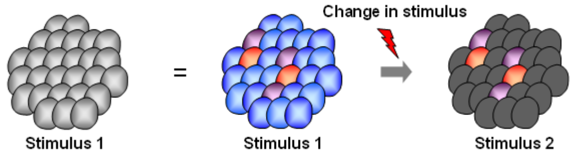
Non-genetic variability and dynamics in expression of key TFs might confer selective advantage as this permits at least some members in a clonal population to respond efficiently to (or survive in) changing conditions

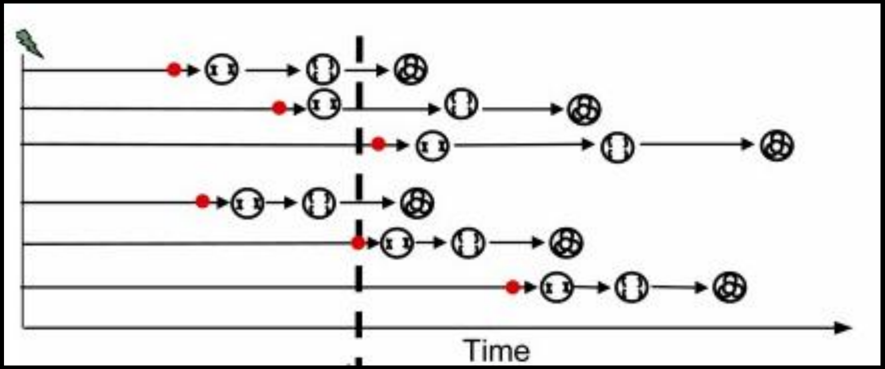
Phenotypic variability in fluctuating environments





Gene network controlling sporulation



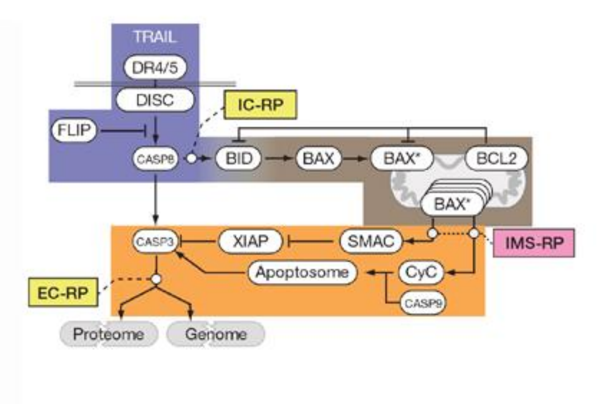


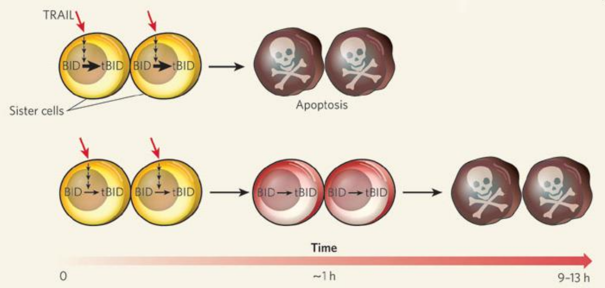
High variability in the expression of top-level TFs in a population of cells may confer a selective advantage as this permits at least some members in a population to respond quickly to changing conditions

Noise in expression of a master regulator of sporulation in yeast

Nachman et al Cell (2008)

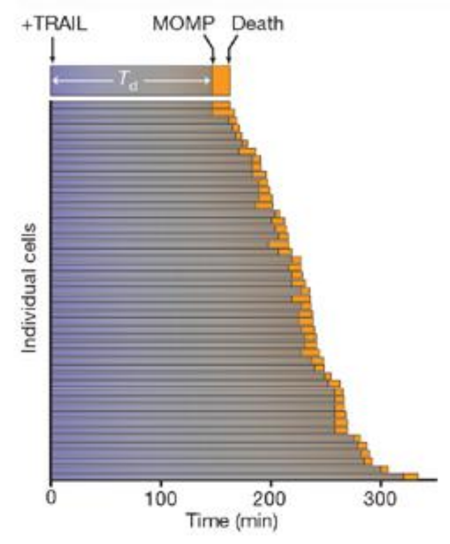
Differential cell-fate outcome in response to uniform stimulus

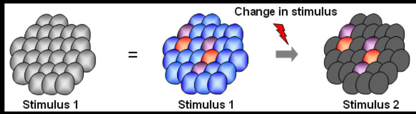




Network controlling apoptosis

Noise in expression of a key regulator of apoptosis in human cancer cells





Spencer et al Nature (2009)
Bastiaens P Nature (2009)

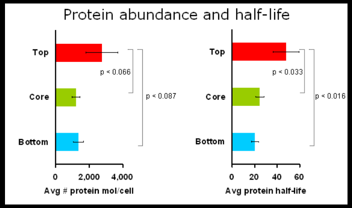
Differences in the levels of proteins regulating apoptosis are the primary causes of cell-to-cell variability in the timing and probability of death in individual members upon induction

Dynamics of the regulatory proteins, which either dictate cell death or survival, varied widely between individual cancer cells upon addition of a drug

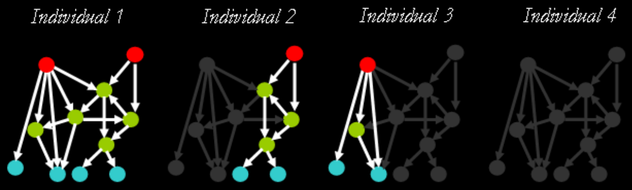
Cohen et al Science (2008)

Summary IV – local dynamics of regulatory networks

Our results suggest that the core- and bottom-level TFs are more tightly regulated at the post-transcriptional level rather than at the transcriptional level itself



Our findings suggest that the interplay between the inherent hierarchy of the network and the dynamics of the TFs permits differential utilization of the same underlying network in distinct members of a population



Jothi R, Balaji S et al.,
***Mol Sys Biol* (2009)**

Conclusions

“Transcriptional networks are made up of network motifs at the local level and have a scale-free structure at the global level”

“Even though close to 90% of the regulatory network in yeast has evolved by duplication, network motifs and scale free structure are not products of duplication events – instead they have been selected for in evolution”

“This has resulted in a network structure that can efficiently re-wire interactions to meet the biological demand placed by the process”

“The interplay between the hierarchy and node dynamics makes the network both robust and adaptable to changing conditions”

“All these aspects of the regulatory network has constrained the way genes are organized across the different linear chromosomes in yeast”

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NCBI, NIH, USA

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EBI, Hinxton, UK

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Teresa Przytycka
NCBI, NIH, USA

Julio Collado-vides
UNAM, Mexico

MRC - Laboratory of Molecular Biology, UK
National Institutes of Health, USA
Schlumberger and Darwin College, Cambridge, UK

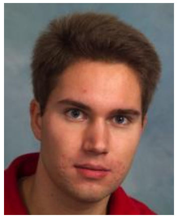
Regulatory Genomics and Systems Biology Group

STRUCTURAL STUDIES

MADAN BABU MOHAN

Regulatory Genomics & Systems Biology

Past and present group members & associate members



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2009

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2010

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2010

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2011

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Rekin's Janky
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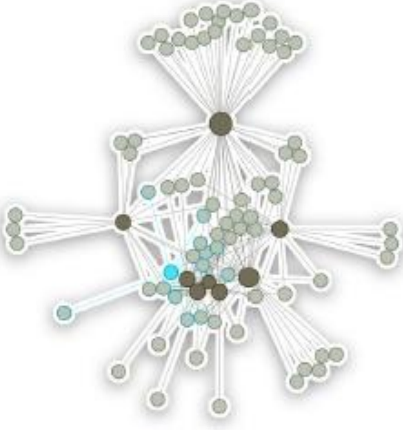
2010

○ **Guilhem Chalancon**, Masters student 2009-2010

○ **Pradeep Kota**, Visiting student 2007

Members of the **Theoretical and Computational Biology** at the LMB group for helpful discussions



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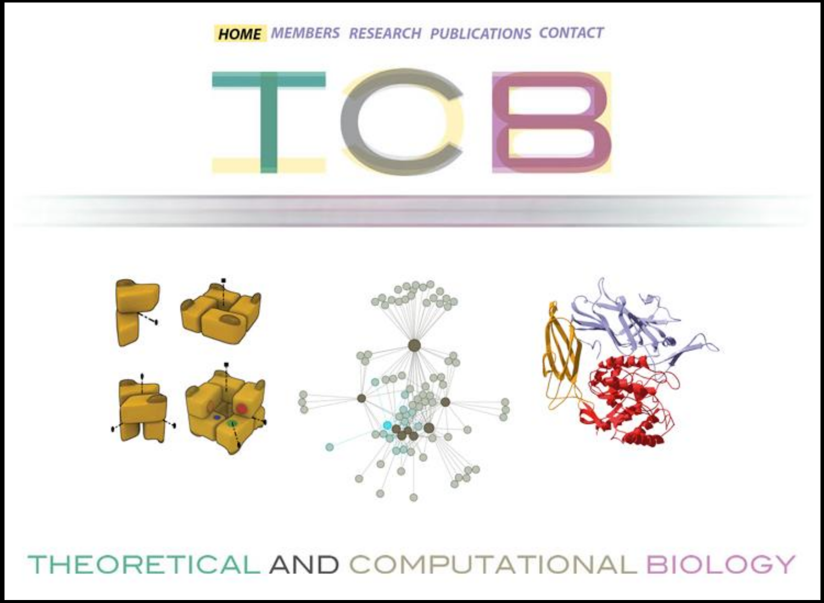

THEORETICAL AND COMPUTATIONAL BIOLOGY

Teichmann

Chothia

Babu

post-doctoral applications invited

madanm@mrc-lmb.cam.ac.uk