Bioinformatics 3

V7 – Gene Regulation

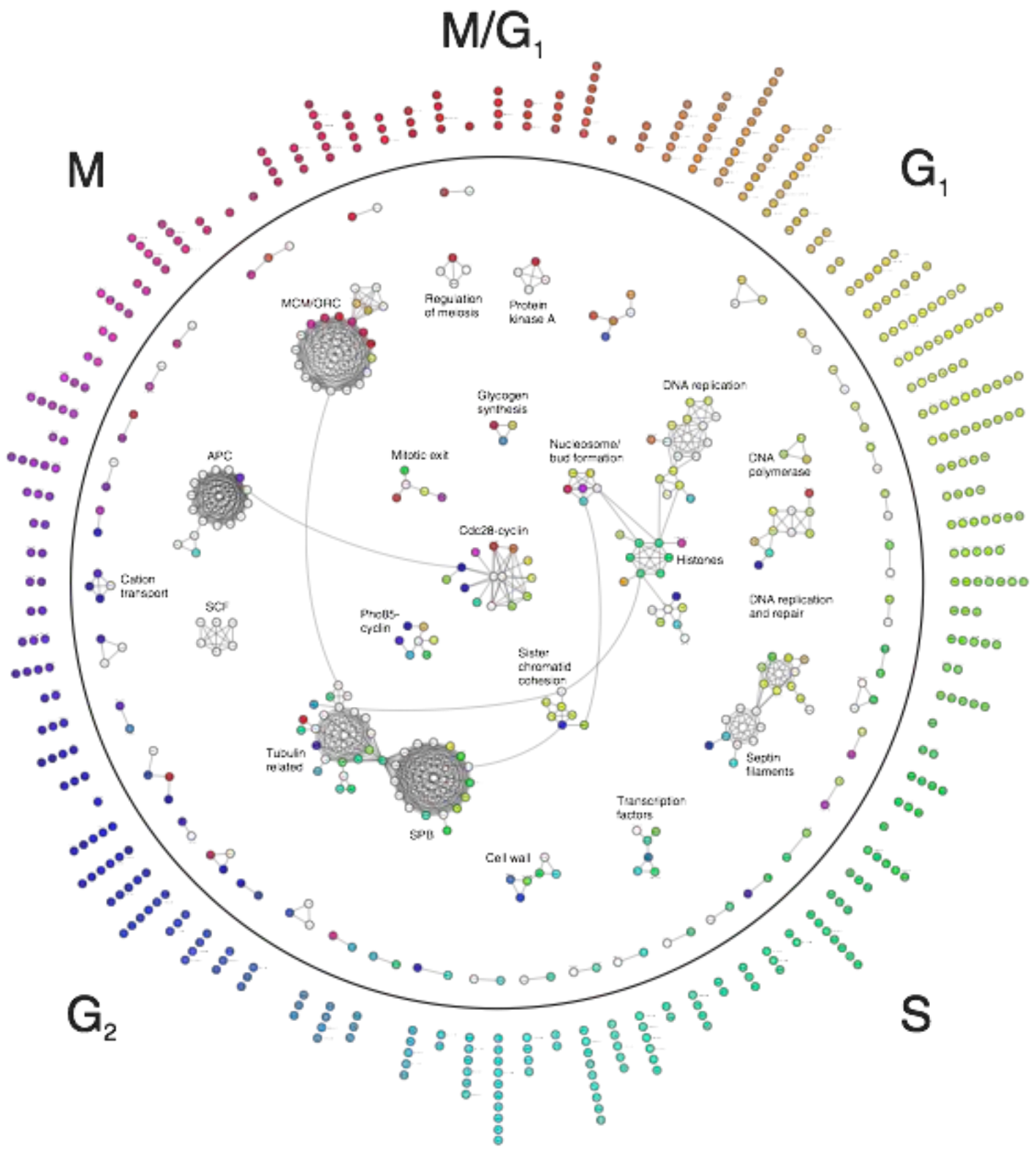
Mon., Nov 11, 2013

Turn, Turn, Turn…

From Lichtenberg et al, Science 307 (2005) 724:

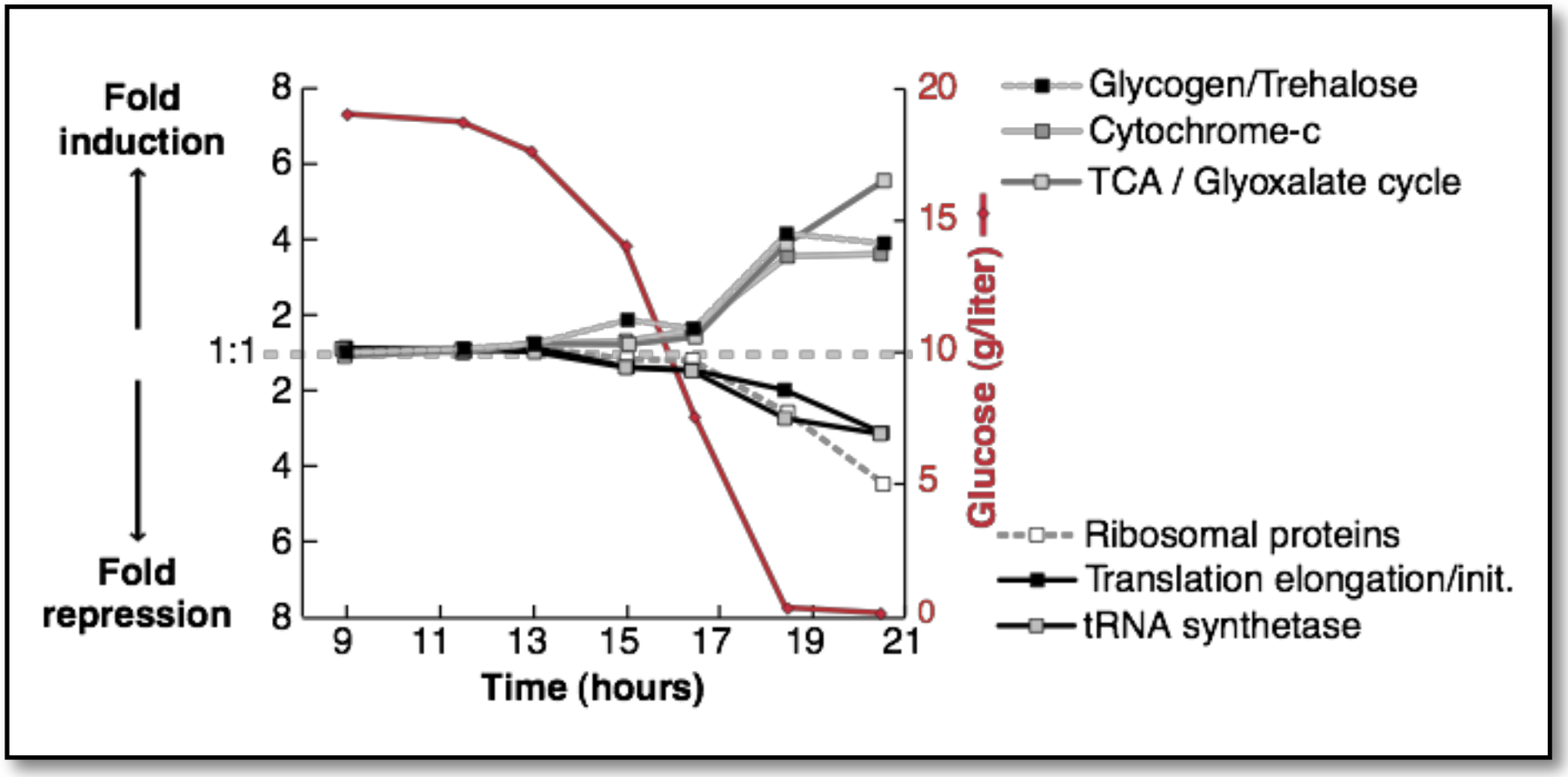
→ certain proteins only occur during well-defined phases in the cell cycle

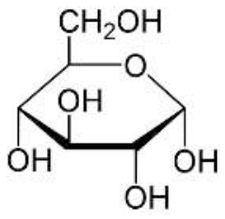
→ how is protein expression regulated?

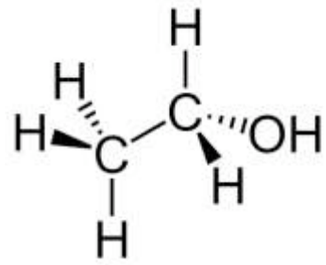


External Triggers

Re-routing of metabolic fluxes during the diauxic shift in *S. cerevisiae*
→ changes in protein abundances (measured via mRNA levels)







anaerobic fermentation:
fast growth on glucose → ethanol

→ **Diauxic shift**

aerobic respiration:
ethanol as carbon source

DeRisi et al., *Science* **278** (1997) 680

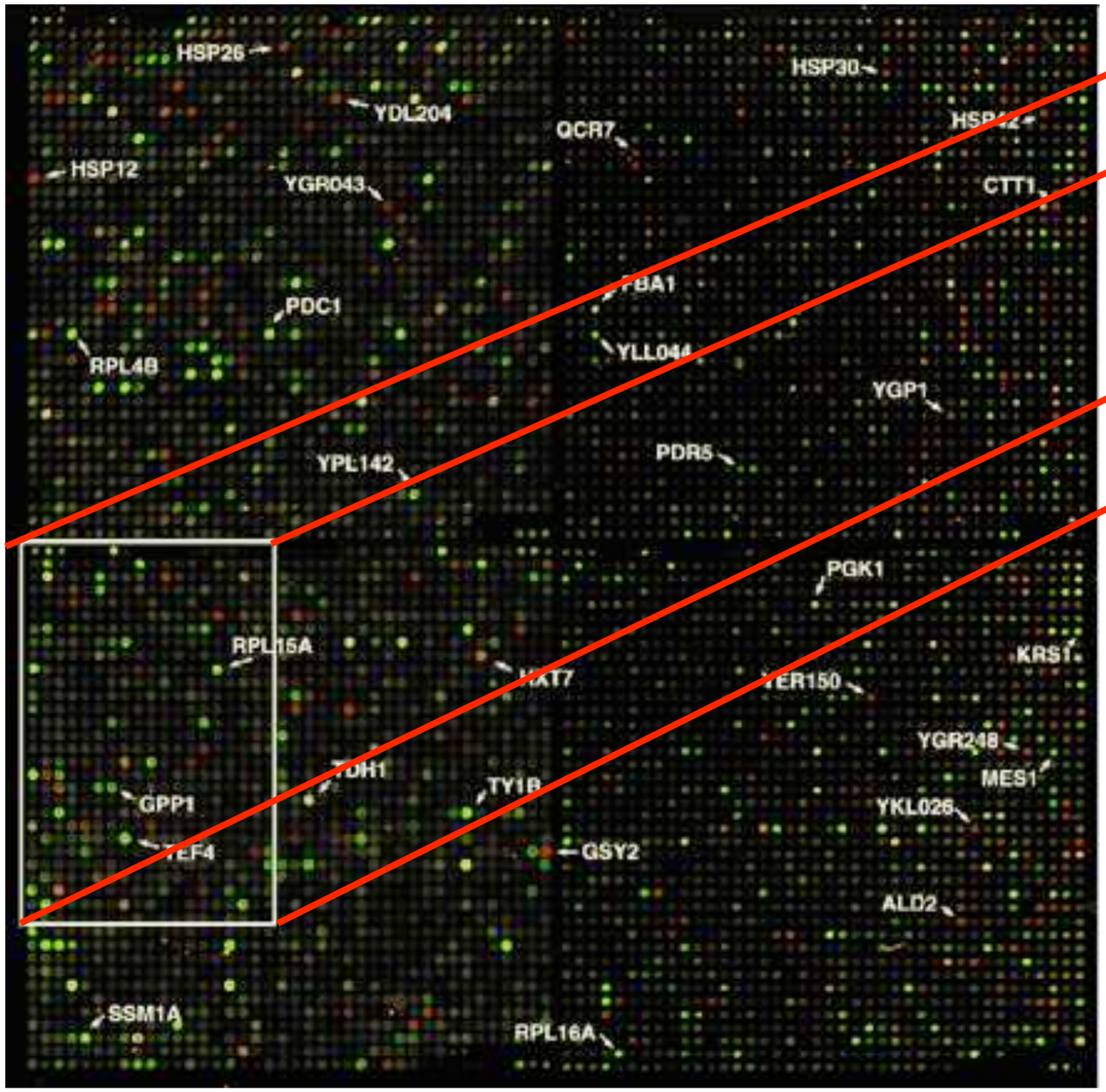
Diauxic shift affects hundreds of genes

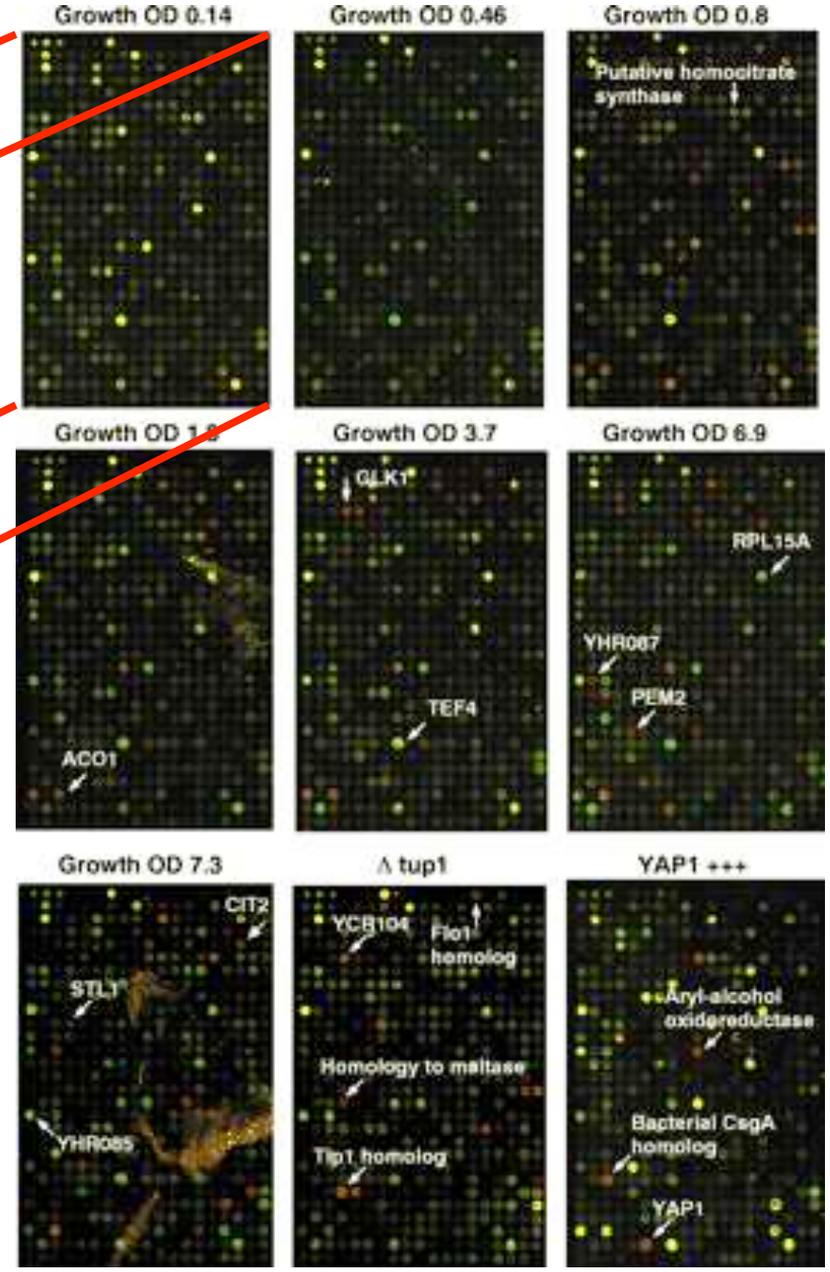
Cy3/Cy5 labels, comparison of 2 probes at 9.5 hours distance; w and w/o glucose

Optical density (OD) illustrates cell growth;

Red: genes induced by diauxic shift (710 genes 2-fold)

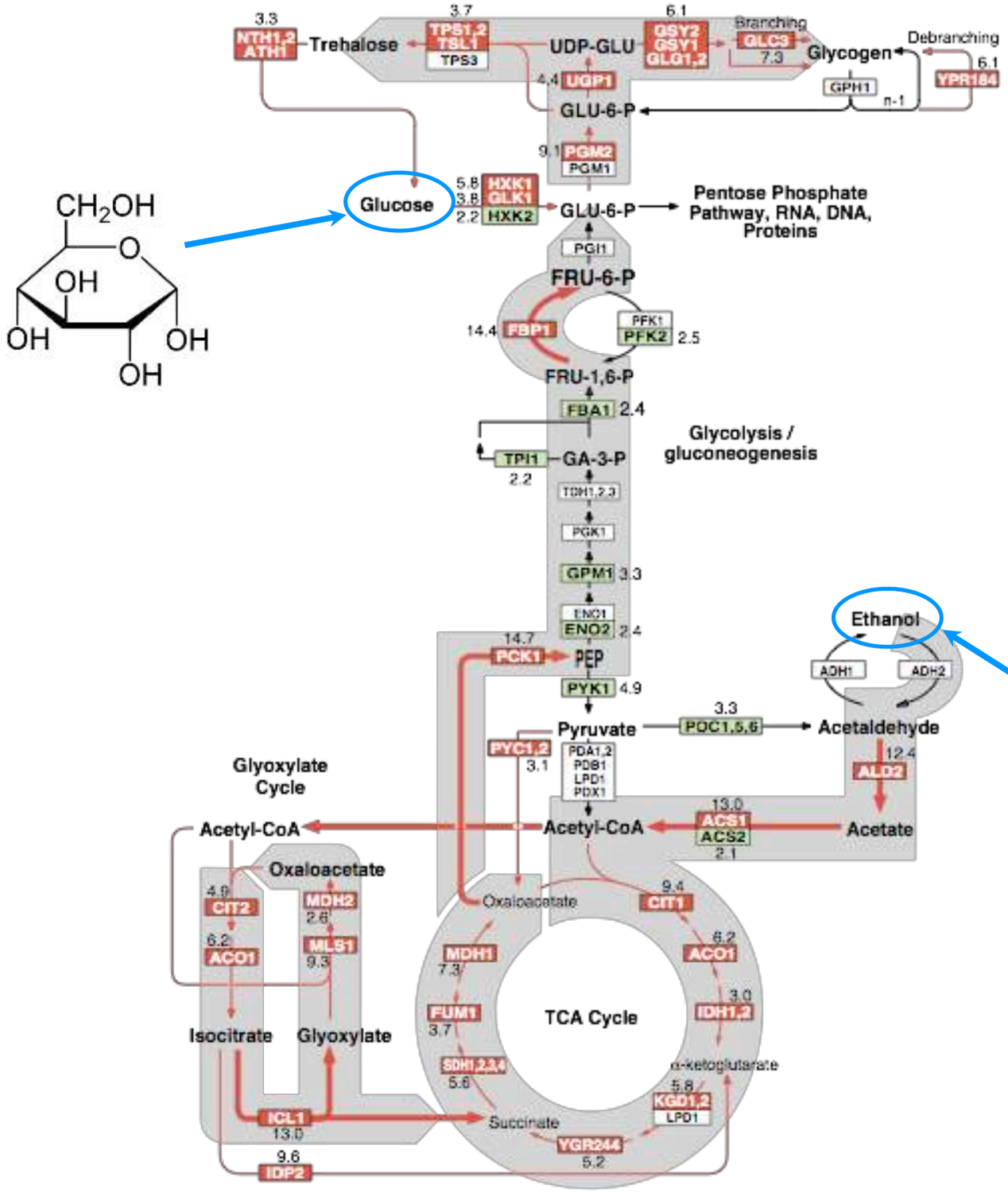
Green: genes repressed by diauxic shift (1030 genes 2-fold





DeRisi et al., *Science* **278** (1997) 680

Flux Re-Routing



during diauxic shift:

fold change

expression increases

expression unchanged

expression diminishes

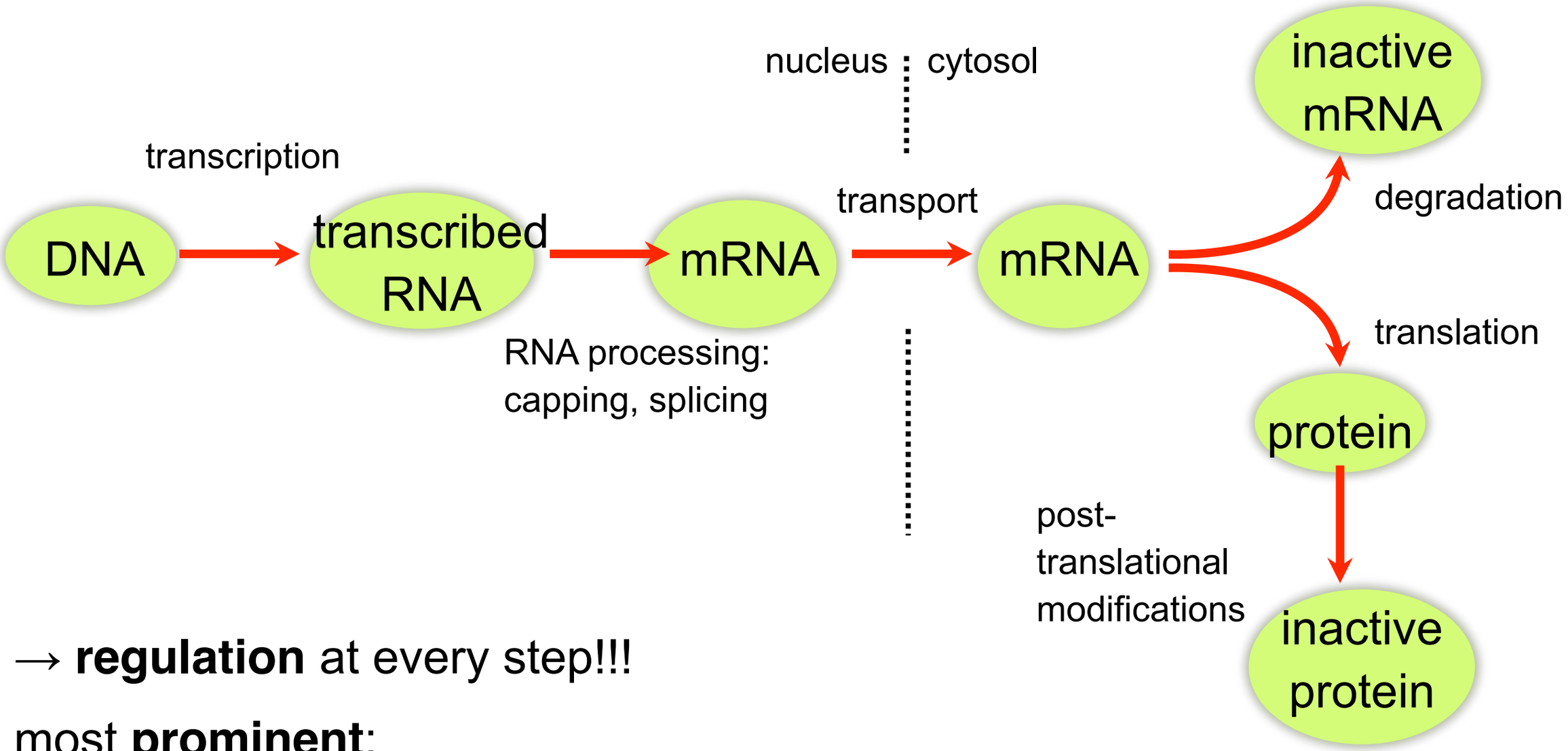
metabolic flux increases

→ **how** are these changes **coordinated?**

DeRisi et al., *Science* **278** (1997) 680

Gene Expression

Sequence of processes: from DNA to functional proteins



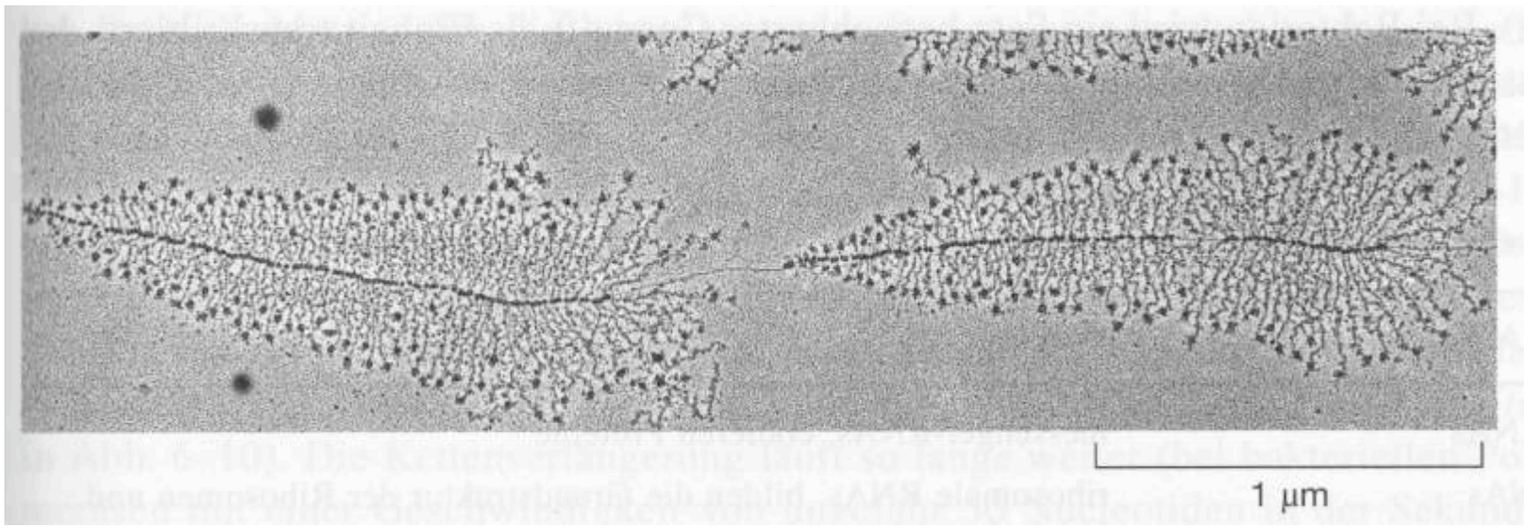
→ **regulation** at every step!!!

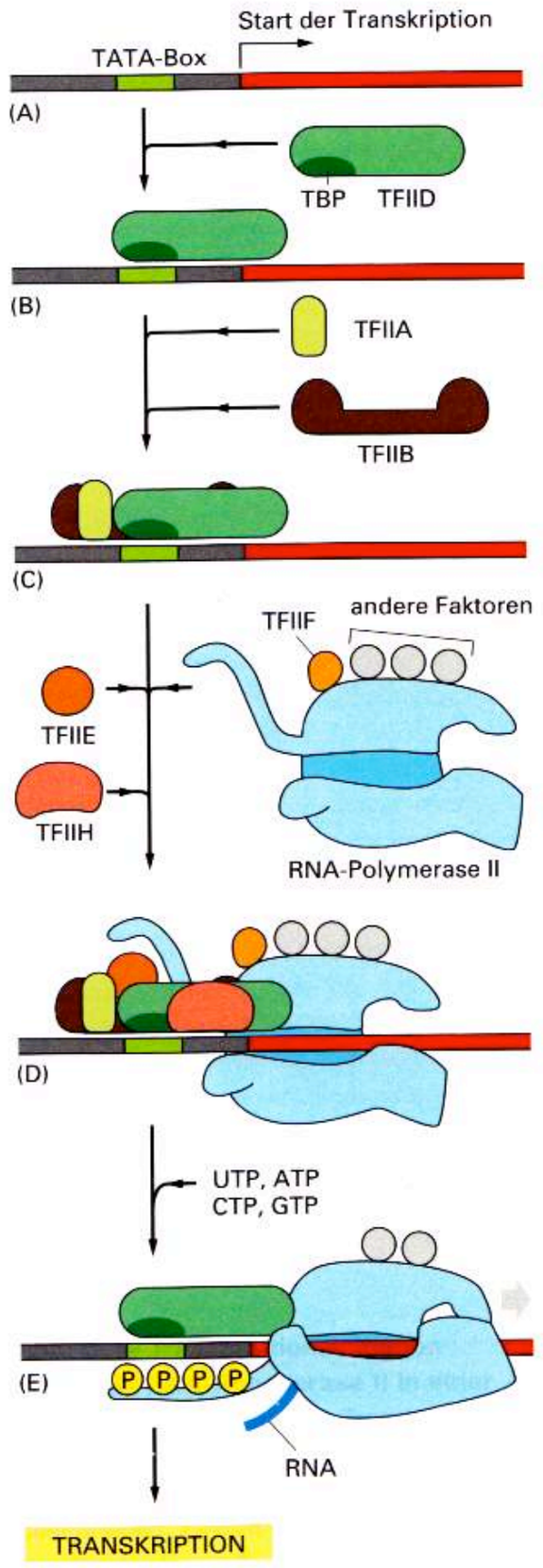
most **prominent**:
activation or repression of the transcription initiation

Transcription Initiation

In eukaryotes:

- several **general** transcription factors **have** to bind
- **specific** enhancers or repressors **may** bind
- then the RNA polymerase binds
- and starts transcription





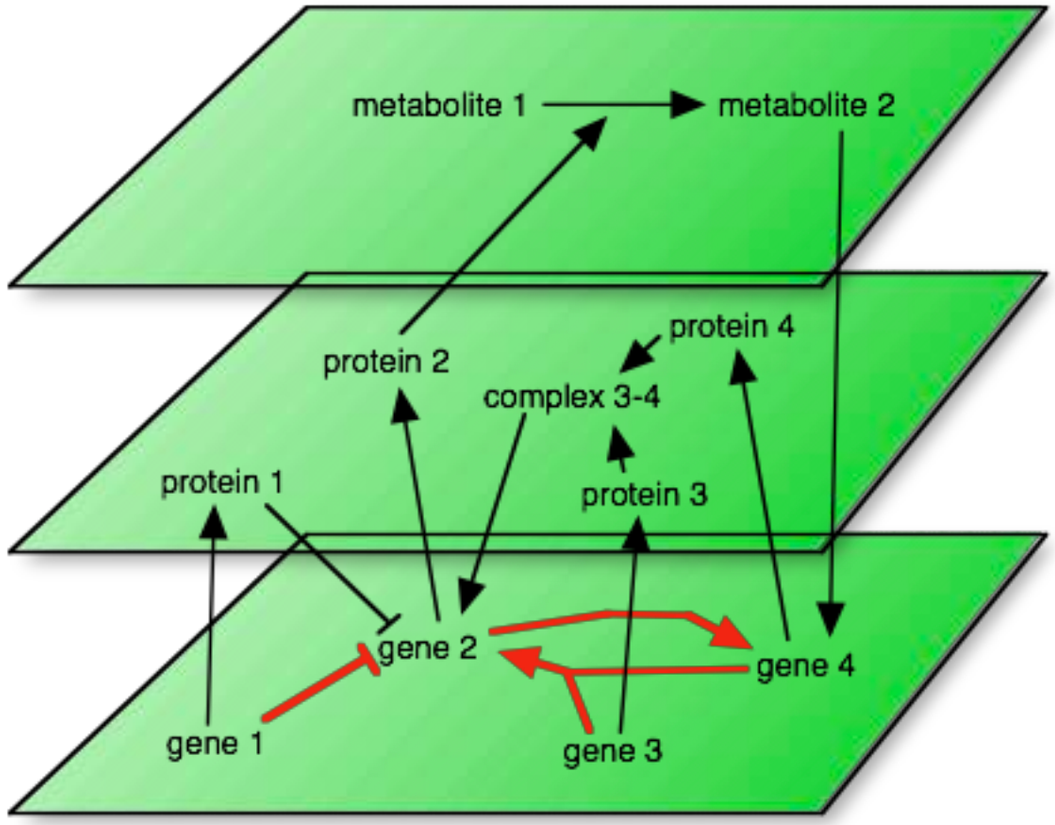
Alberts et al.
"Molekularbiologie der Zelle", 4. Aufl.

Layers upon Layers

Biological regulation
via proteins and metabolites

<=>

Projected regulatory network



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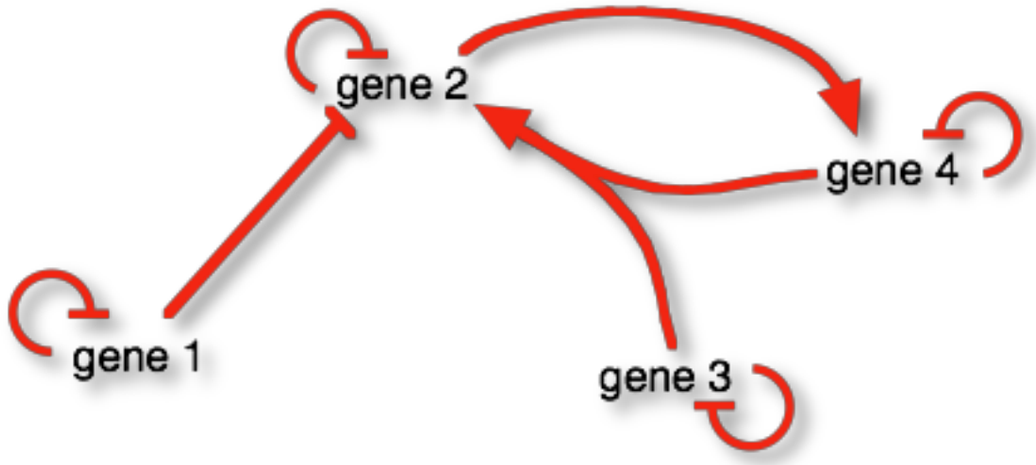


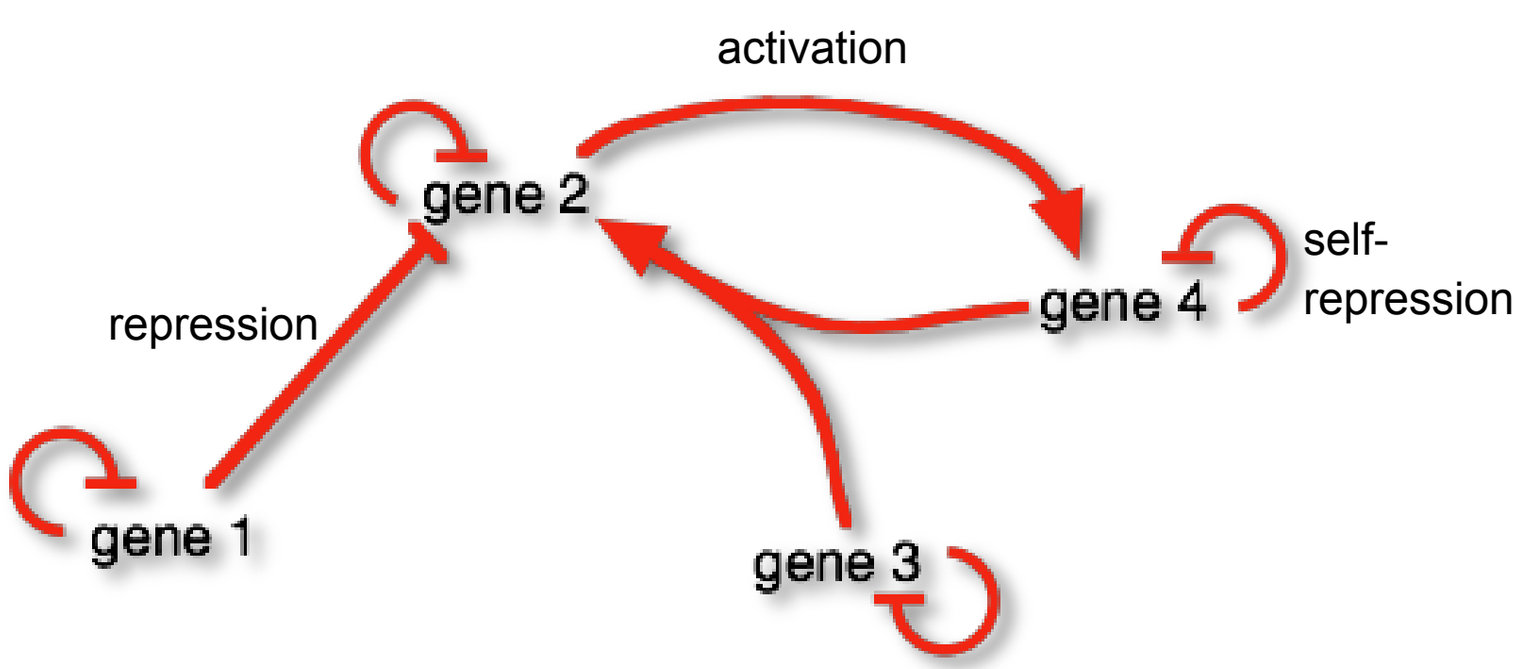
Remember:
genes do not interact directly

Conventions for GRN Graphs

Nodes: genes that code for proteins which catalyze products …
→ everything projected onto respective gene

Gene regulation networks have "cause and action"
→ **directed** networks

A gene can enhance or suppress the expression of another gene
→ **two types** of arrows



Quorum sensing in bacteria

Quorum sensing is a system of stimulus and response correlated to population density.

Many species of bacteria use quorum sensing to coordinate gene expression according to the density of their local population.

They release so-called **autoinducer molecules** (e.g. homo-serine lactone or HSL) to their environment. These may be taken up by other bacteria nearby. In this way, the autoinducer concentration reflects the population density.

Bacteria use quorum sensing to **coordinate certain behaviors** such as

- biofilm formation,
- virulence, and
- antibiotic resistance,

based on the local density of the bacterial population.

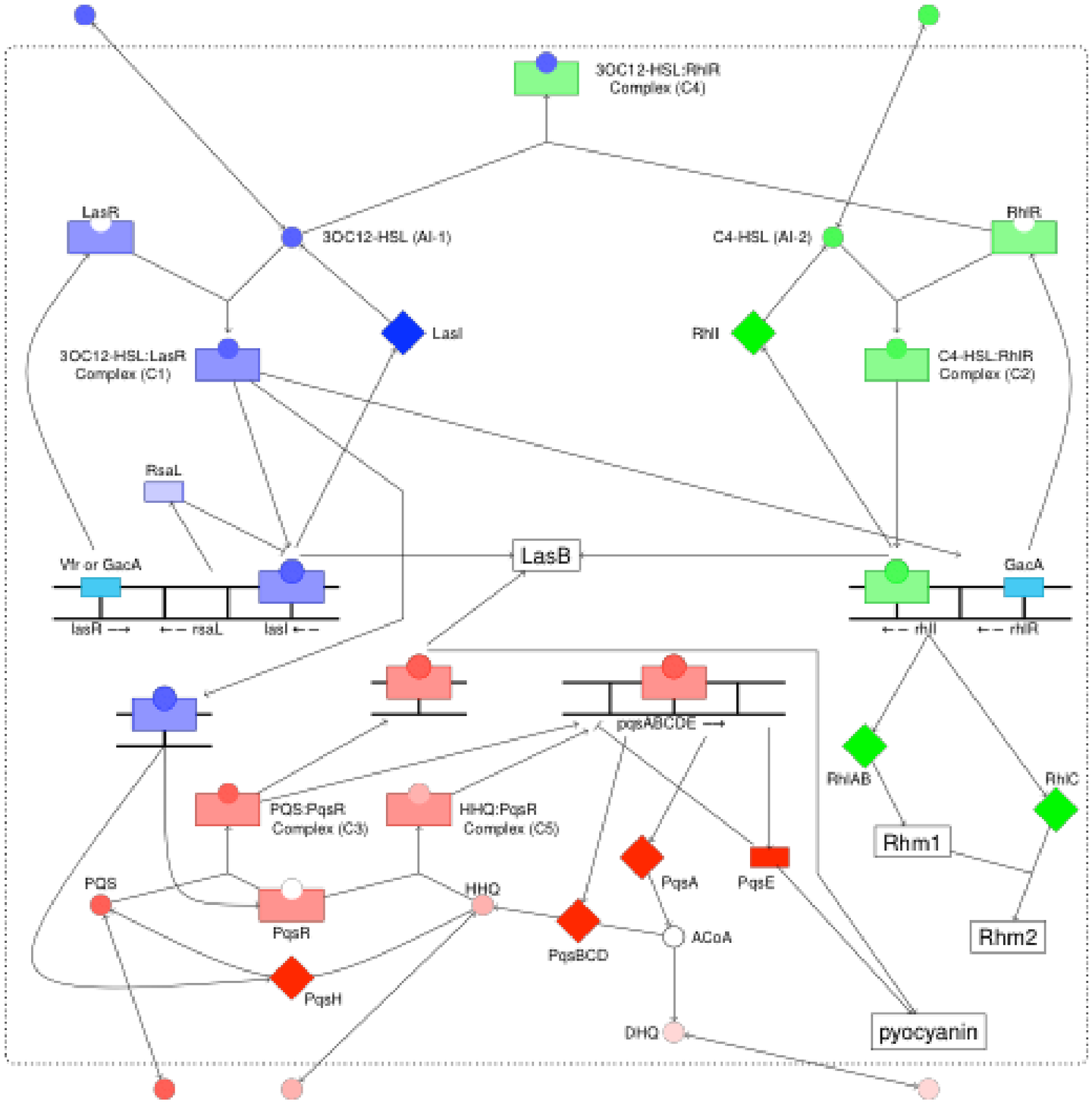
www.wikipedia.org

Quorum Sensing in *P. aeruginosa*

In *P. aeruginosa*, the QS network consists of 3 systems termed *las*, *rhl*, and *pqs* that are organized hierarchically.

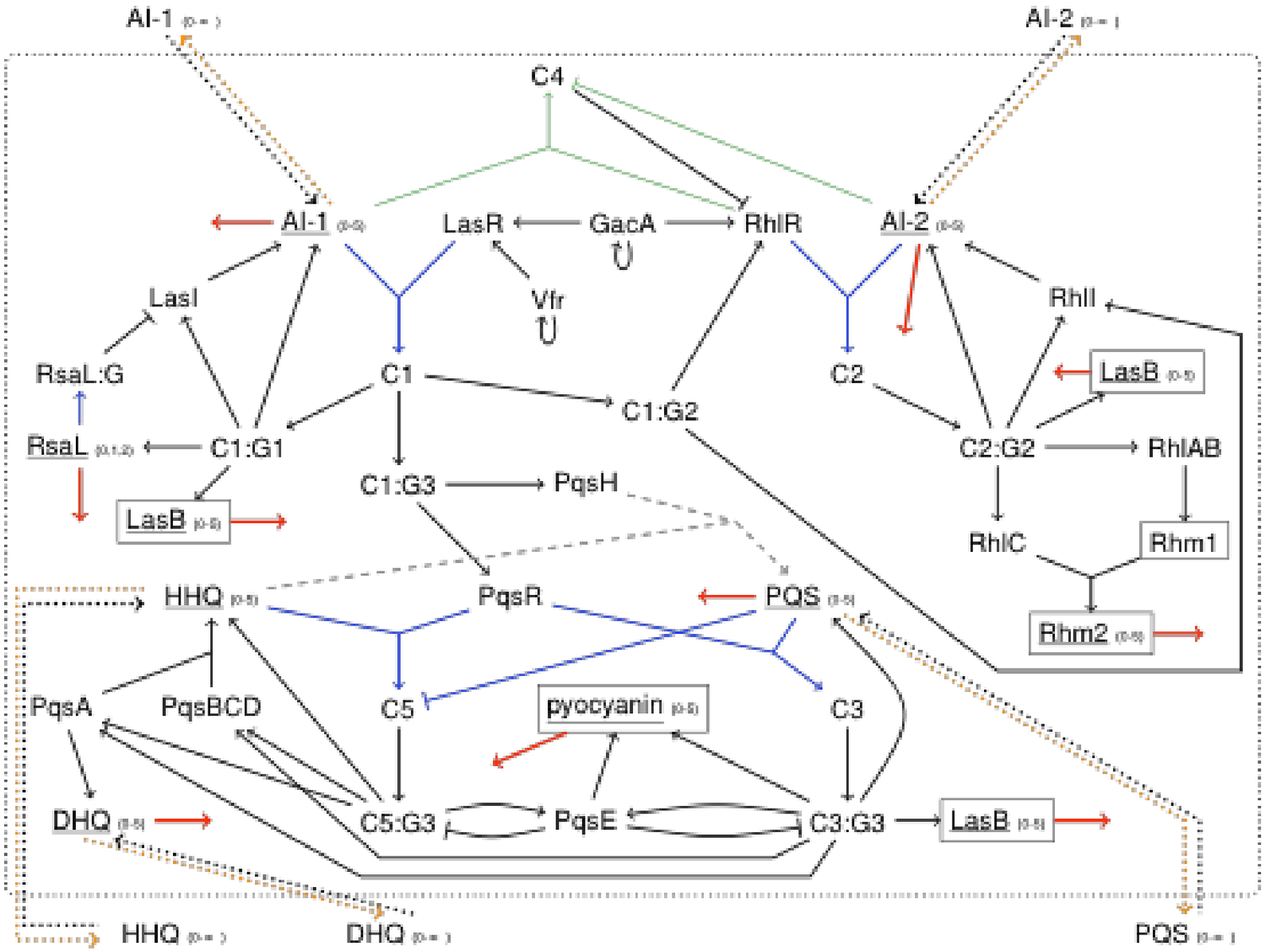
Selectively targeting the QS machinery by signaling molecule inhibitors may avoid development of resistance mutations.

Aim: develop simple computational model that can account for effects of small-molecule inhibitors and resistance mutations.



Schaadt et al. BMC Systems Biol. (2013)

QS network as a generalized Boolean topology



Nodes named C represent a complex between autoinducer and receptor, C:G is the complex bound to an operon.

black edge = threshold is 1

blue edge = state of underlined node must be ≥ 2;

orange edge = state of underlined node must be ≥ 3;

thin **green** edge = state of underlined node must be ≥ 4;
numbers denote possible states for a node;

dotted arrows : transport processes

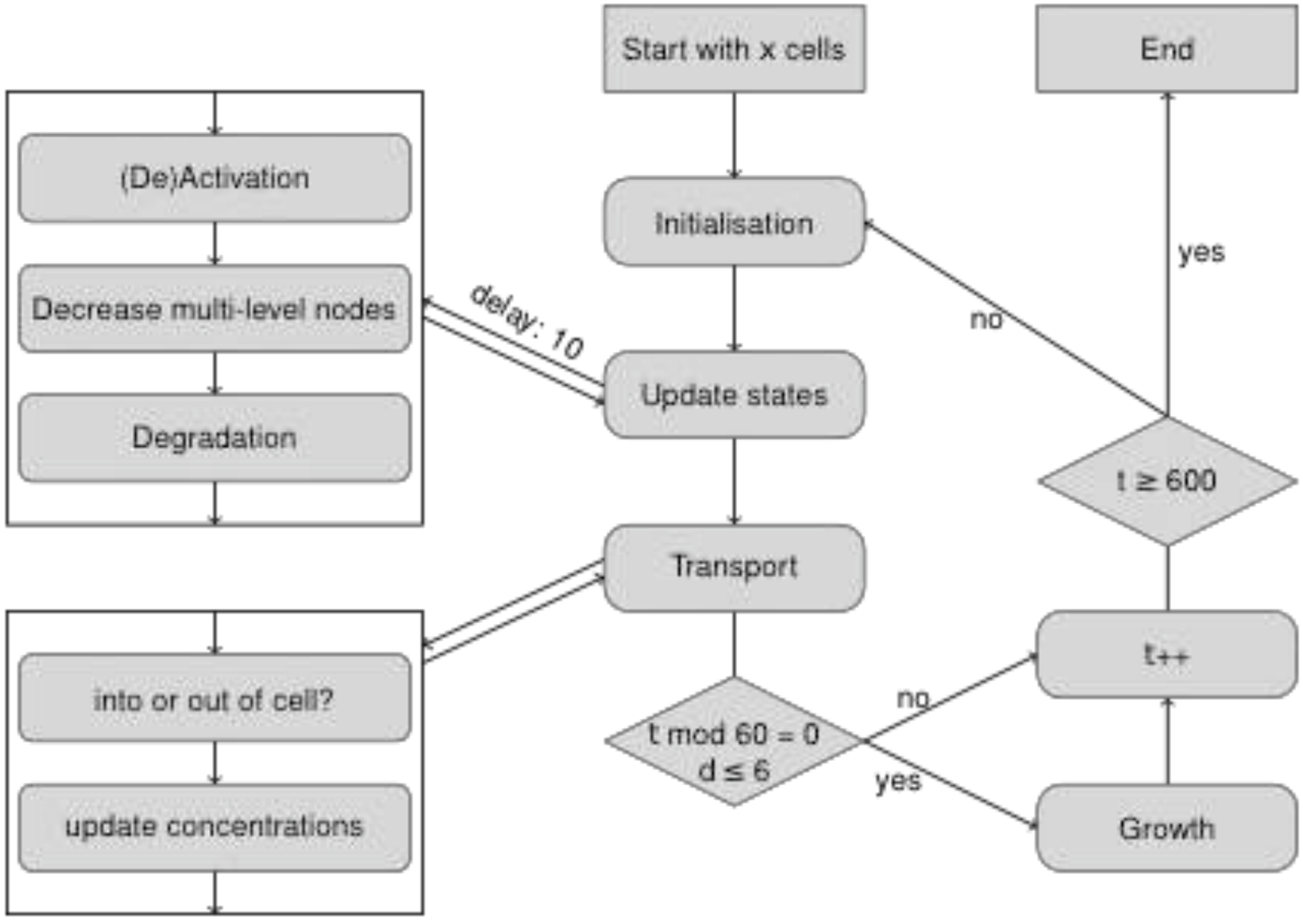
thick **red** edge : happens after a certain number of time steps (degradation).

dashed **grey** arrows : reaction that occurs by chance with a certain probability.

Reactions in the QS systems

Reaction	Type	Reference
GacA → LasR	activation, transcription + translation	Reimmann et al. (1997) Mol. Microbiol.
GacA → RhlR	activation, transcription + translation	Reimmann et al. (1997) Mol. Microbiol.
Vfr → LasR	activation, transcription + translation	Albus et al. (1997) J Bacteriol.
AI-1 + LasR → C1	association	Seed et al. (1995) J Bacteriol.
C1 → C1:G1	activation	Seed et al. (1995) J Bacteriol.
C1:G1 → LasI	transcription + translation	Seed et al. (1995) J Bacteriol.
C1:G1 → RsaL	transcription + translation	de Kievit et al. (1999) J Bacteriol.
RsaL → RsaL:G	activation	de Kievit et al. (1999) J Bacteriol.
RsaL:G −| LasI	inhibition	de Kievit et al. (1999) J Bacteriol.
LasI → AI-1	enzymatic reaction (formation)	Passador et al. (1993) Science
AI-1 + RhlR → C4	association	Pesci et al. (1997) J Bacteriol.
C1 → C1:G2	activation	Pesci et al. (1997) J Bacteriol.
C1:G2 → RhlR	transcription + translation	Pesci et al. (1997) J Bacteriol.
AI-2 −| C4	blocking	Pesci et al. (1997) J Bacteriol.
C1:G2 → RhlI	transcription + translation	- - - - -
AI-2 + RhlR → C2	association	Ochsner and Reiser (1995) PNAS
C2 → C2:G2	activation	Ochsner and Reiser (1995) PNAS
C2:G2 → RhlI	transcription + translation	Ochsner and Reiser (1995) PNAS
RhlI → AI-2	enzymatic reaction (formation)	Ochsner and Reiser (1995) PNAS
C1 → C1:G3	activation	Rampioni et al. (2010) Environ. Microbiol.
C1:G3 → PqsR	transcription + translation	Rampioni et al. (2010) Environ. Microbiol.
C1:G3 → PqsH	transcription + translation	Rampioni et al. (2010) Environ. Microbiol.
HHQ + PqsH → PQS	enzymatic reaction (formation)	Deziel et al. (2004) PNAS
PQS + PqsR → C3	association	Deziel et al. (2004) PNAS
C3 → C3:G3	activation	Deziel et al. (2004) PNAS
C3:G3 → PqsABCDE	transcription + translation	Deziel et al. (2004) PNAS
PqsA + PqsBCD → HHQ	enzymatic reaction (formation)	Deziel et al. (2004) PNAS
HHQ + PqsR → C5	association	Xiao et al. (2006) Mol. Microbiol.
C5 → C5:G3	activation	Xiao et al. (2006) Mol. Microbiol.
C5:G3 → PqsABCDE	transcription + translation	Xiao et al. (2006) Mol. Microbiol.
PqsA → DHQ	enzymatic reaction (formation)	

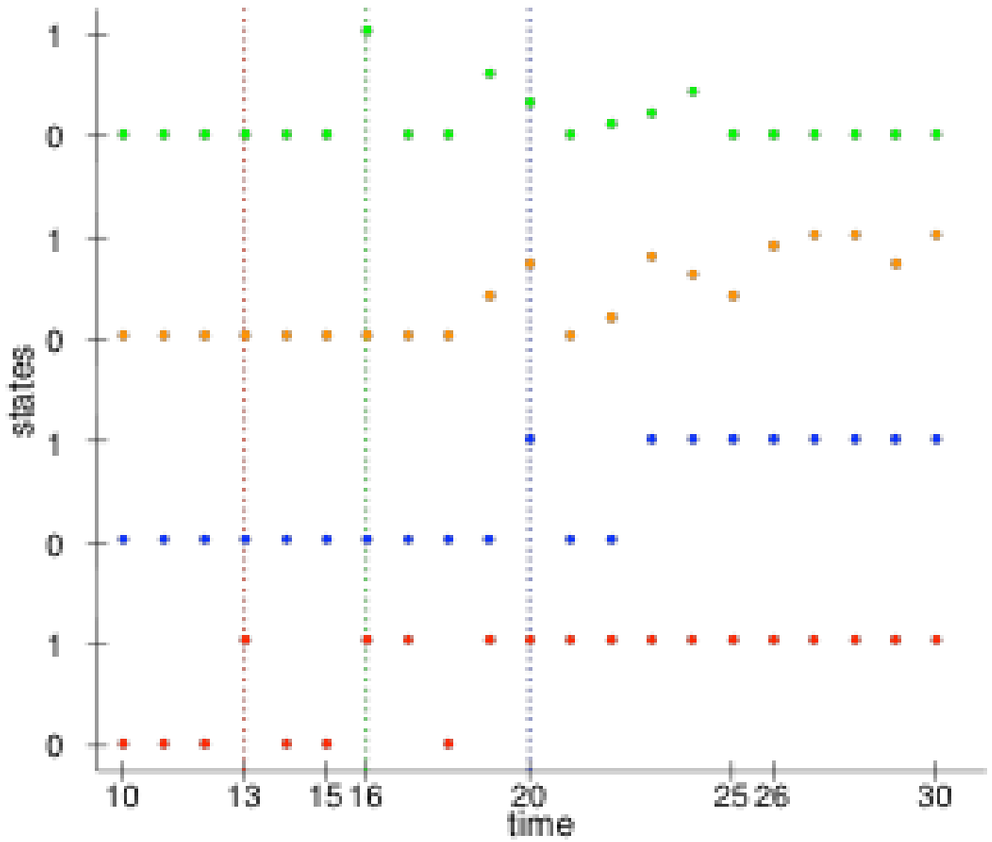
Network propagation



Sample trajectory

time step	HHQ	PQS	C3	C5	C3:G3	C5:G3	PqsA	PqsBCD	PqsE
10	0	0	0	0	1	1	0	0	0
11	1	1	0	0	0	0	1	1	1
12	2	1	0	0	0	0	0	0	0
13	2	1	0	0	0	0	0	0	0
14	2	1	0	0	0	0	0	0	0
15	2	1	0	0	0	0	0	0	0
16	0	2	0	1	0	0	0	0	0
17	0	2	0	0	0	1	0	0	0
18	1	2	0	0	0	0	1	1	1
19	2	1	1	0	0	0	0	0	0
20	0	1	0	1	1	0	0	0	0
21	0	2	0	0	0	1	1	1	1
22	2	1	1	0	0	0	1	1	1
23	2	1	0	1	0	0	0	0	0
24	0	2	0	1	0	1	0	0	0
25	1	1	1	0	0	1	1	1	1
26	2	2	0	0	0	0	1	1	1
27	1	2	1	0	0	0	0	0	0
28	0	2	1	0	1	0	0	0	0
29	0	2	1	0	1	0	1	1	1
30	1	2	1	0	0	0	1	1	1
31	1	2	1	0	0	0	0	0	0
32	0	2	1	0	1	0	0	0	0
33	0	2	1	0	1	0	1	1	1
34	1	2	1	0	0	0	1	1	1
35	2	1	1	0	0	0	0	0	0

Simulation start



Based on the complexes using minimal initial conditions.

Red: first complex of AI–1 and LasR

Blue: second complex of AI–2 and RhlR.

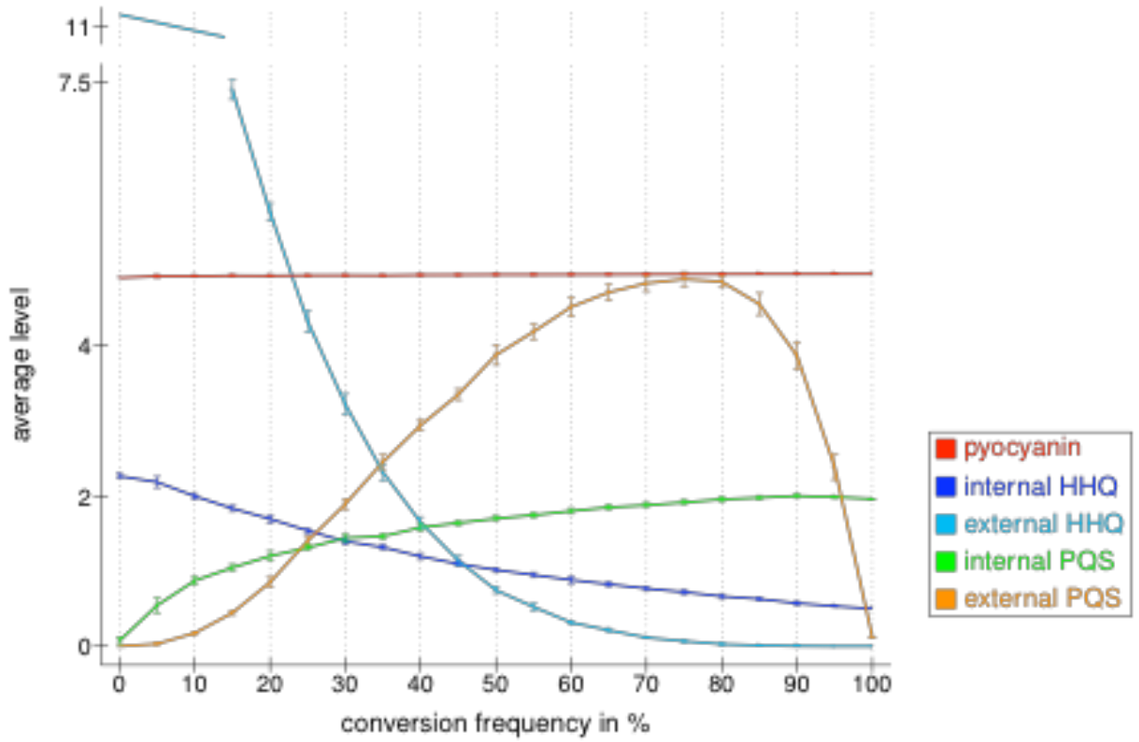
Orange: complex C3 of the *pqs* system between PQS and PqsR

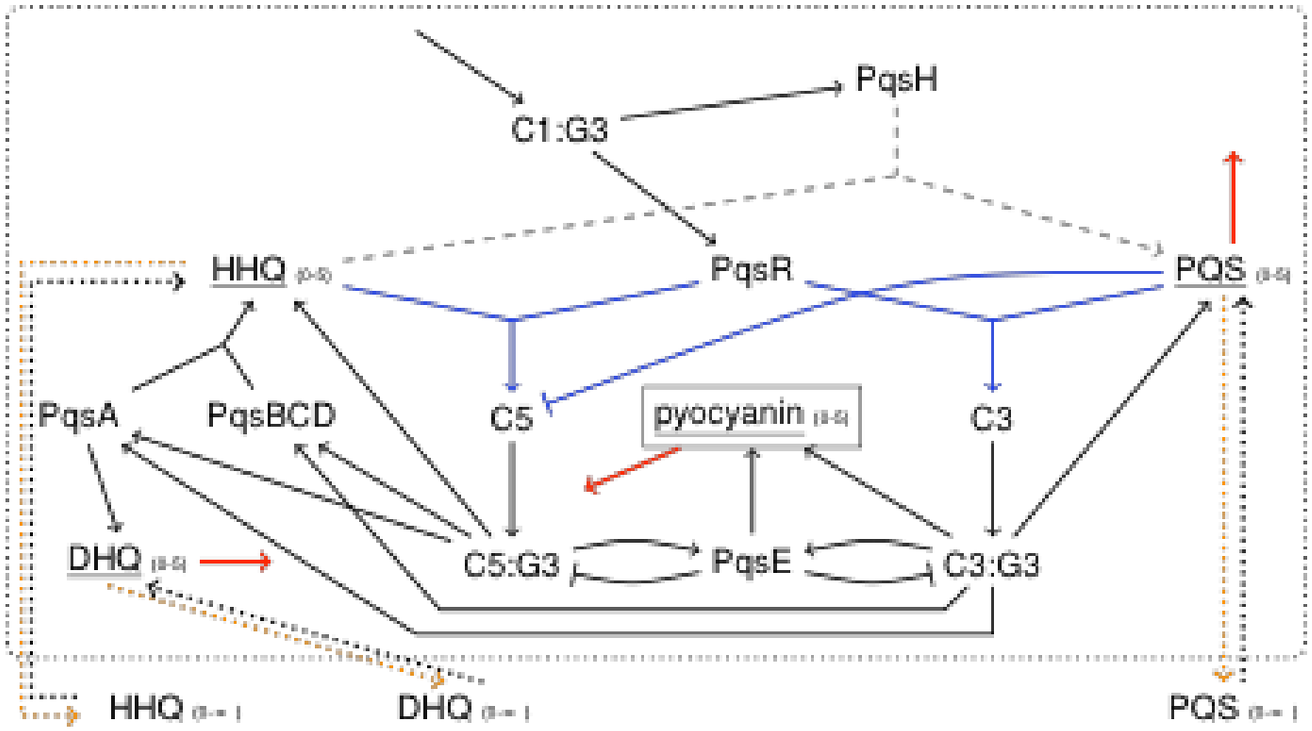
Green: complex C5 between HHQ and PqsR.

Effect of the PQS production rate

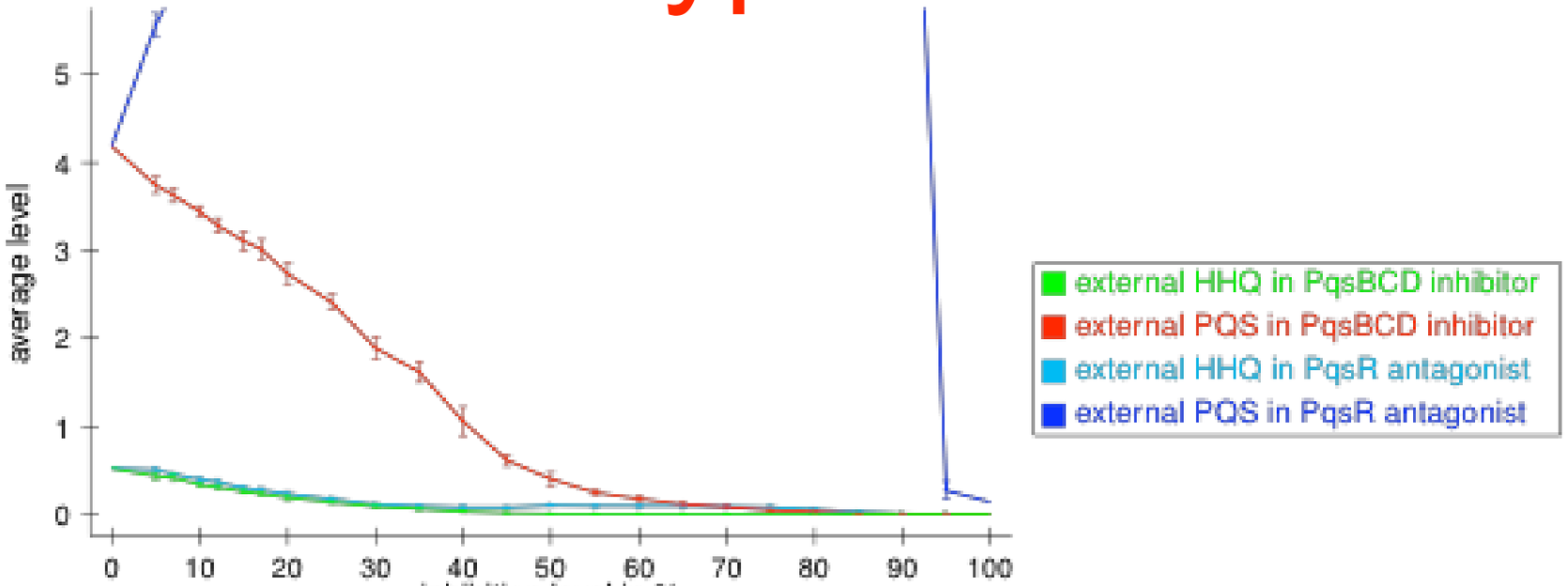
Study effect of PQS production rate on the average levels of autoinducers HHQ, PQS, and pyocyanin.

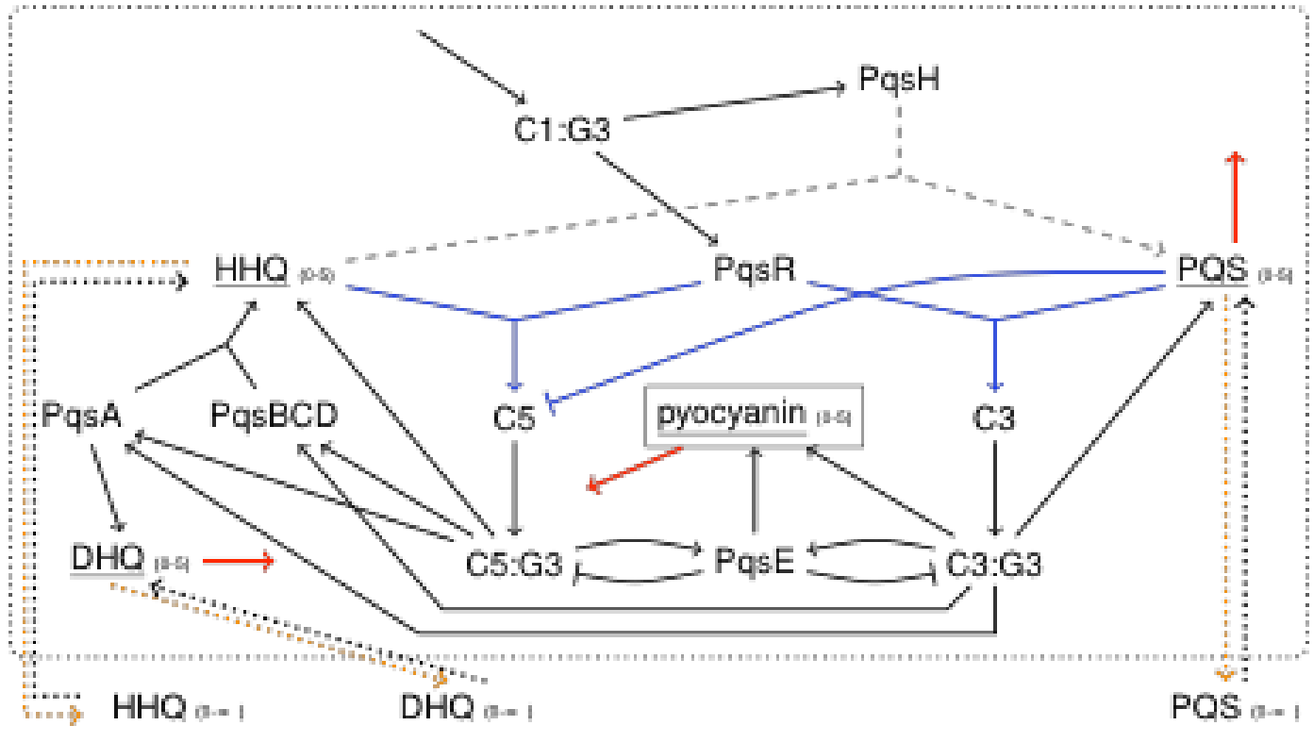
Conversion frequency: probability per Iteration step that PQS is produced by PqsH.





Calculated PQS and pyocyanin levels for wild type and knock–out mutants





Study effect of PqsBCD inhibitors and PqsR antagonists on the average external levels of autoinducers HHQ, PQS.

Summary

Results

- rule–based simulations fulfill the behavior expected from literature considering the external level of autoinducers.
- In the presence of PqsBCD inhibitors, the external HHQ and PQS levels are indeed clearly reduced. The magnitude of this effect strongly depends on the inhibition level.
- It seems that the pyocyanin pathway is incomplete.

Conclusions

- To match experimental observations we suggest a modified network topology in which PqsE and PqsR act as receptors and an autoinducer as ligand that up–regulate pyocyanin in a concerted manner.
- While the PQS biosynthesis is more appropriate as target to inhibit the HHQ and PQS formation, blocking the receptor PqsR that regulates the biosynthesis reduces the pyocyanin level stronger.

E. coli Regulatory Network

BMC Bioinformatics

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Research article

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Hierarchical structure and modules in the *Escherichia coli* transcriptional regulatory network revealed by a new top-down approach

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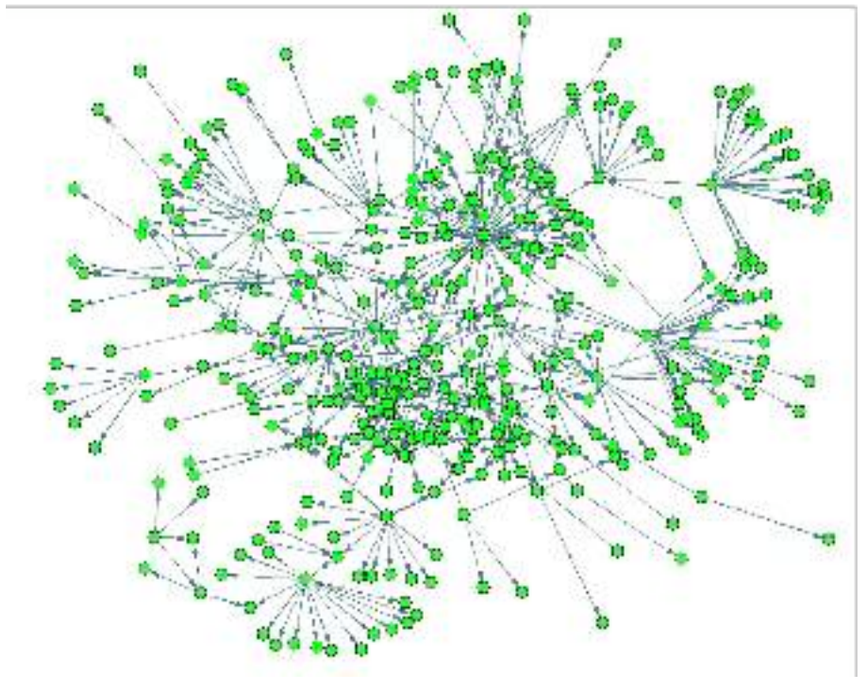
BMC Bioinformatics **5** (2004) 199

Hierarchies

Largest WCC: 325 operons (3/4 of the complete network)

WCC = weakly connected component (ignore directions of regulation)



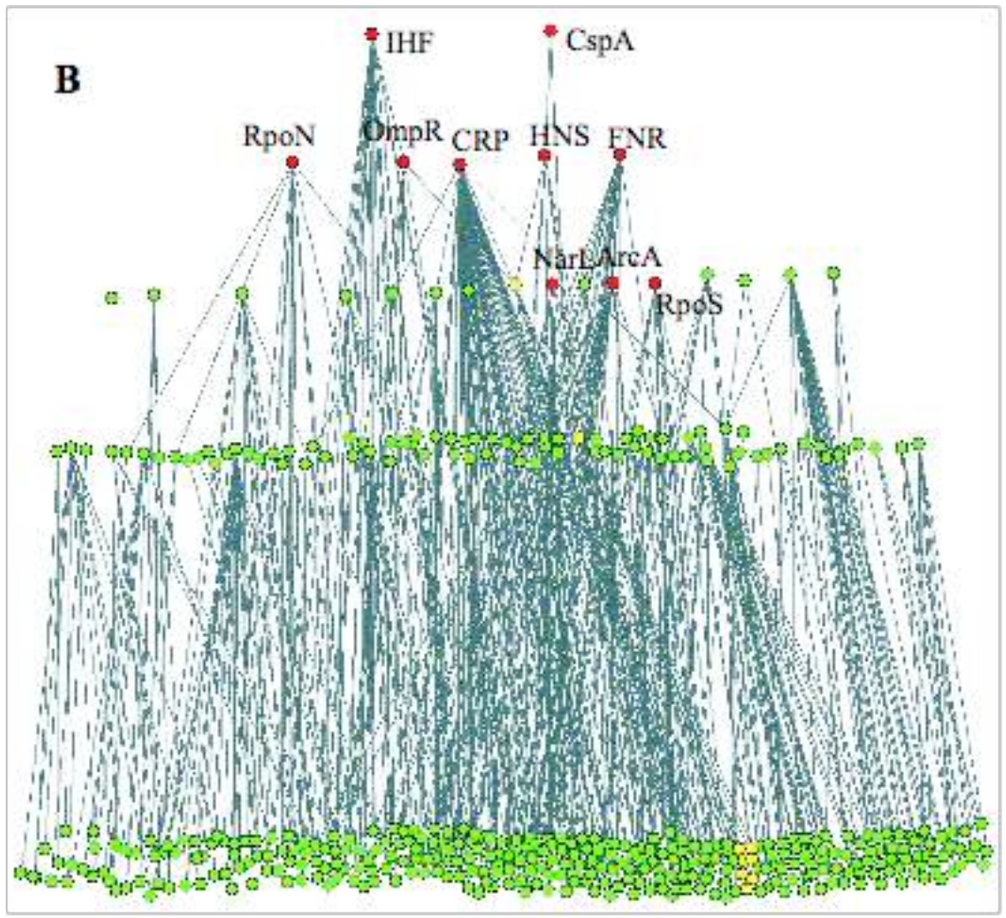
Network from standard layout algorithm

→

Lowest level: operons that code for TFs with only auto-regulation, or no TFs

Next layer: delete nodes of lower layer, identify TFs that do not regulate other operons in this layer (only lower layers)

Continue …



Network with all regulatory edges pointing downwards

→ a few global regulators (•) control all the details

Ma et al., *BMC Bioinformatics* **5** (2004) 199

Global Regulators in *E. coli*

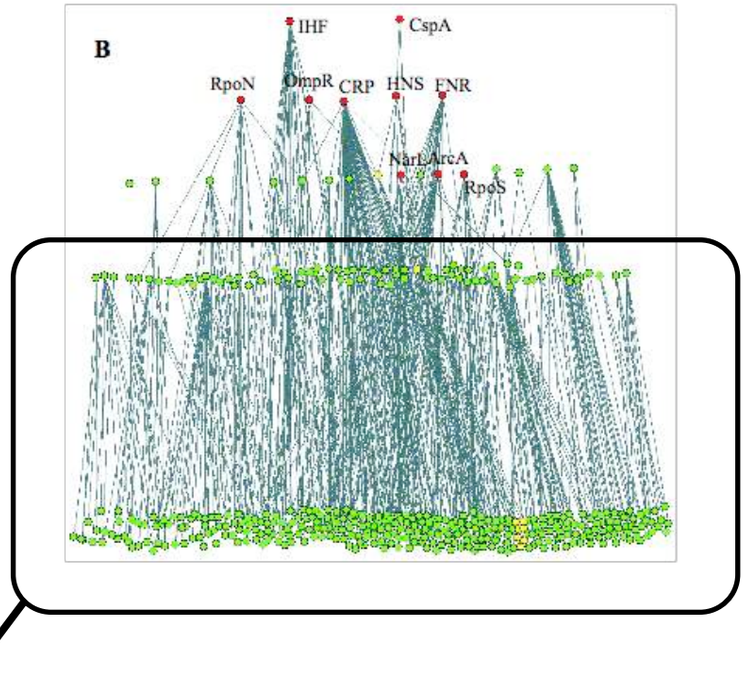
Table 1: Global regulators and their regulated operons and functions in the regulatory network of *E. coli*.

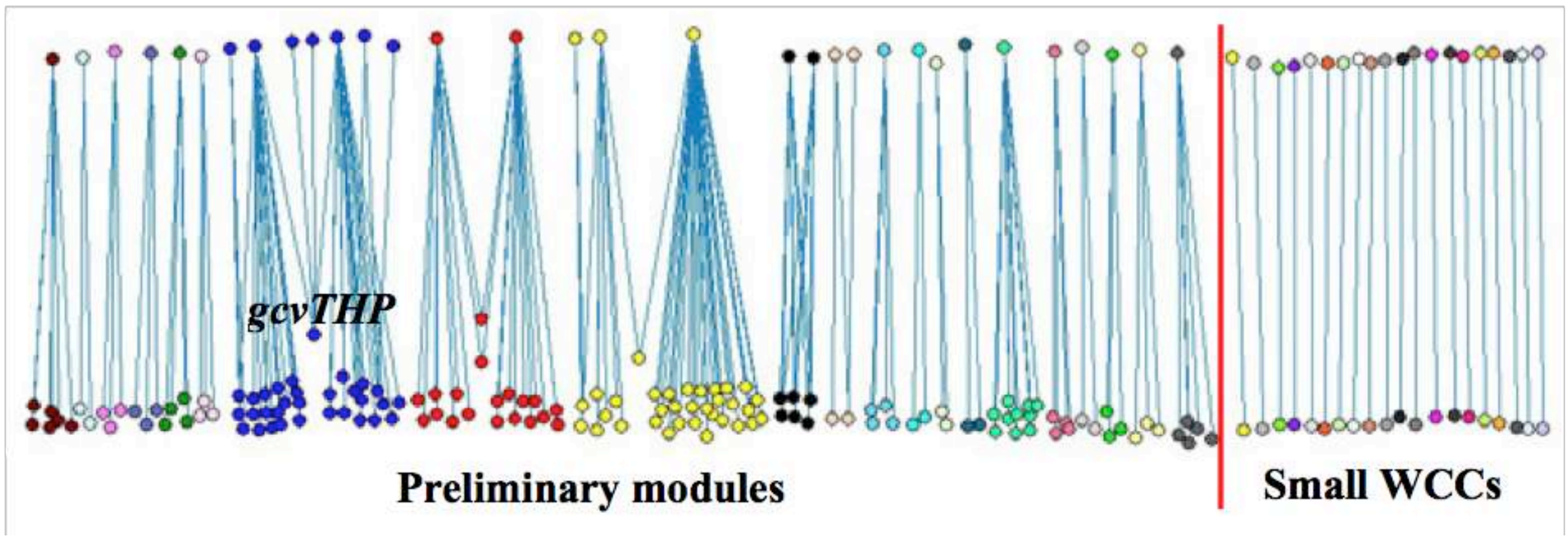
Global regulator	directly regulated Operons	Total regulated operons	Modules regulated	Function
IHF	21	39	15	integration host factor
CspA	2	24	5	Cold shock protein
CRP	72	112	21	cAMP receptor protein
FNR	22	38	16	anaerobic regulator, regulatory gene for nitrite and nitrate reductases, fumarate reductase
HNS	7	22	5	DNA-binding global regulator; involved in chromosome organization; preferentially binds bent DNA
OmpR	6	20	3	Response regulator for osmoregulation; regulates production of membrane proteins
RpoN	12	17	4	RNA polymerase sigma 54 subunit
RpoS	14	24	8	stationary phase sigma factor
ArcA	20	21	6	Response regulator protein represses aerobic genes under anaerobic growth conditions and activates some anaerobic genes
NarL	13	15	5	Two-component regulator protein for nitrate/nitrite response

Ma et al., *BMC Bioinformatics* **5** (2004) 199

Modules

Remove top 3 layers and determine WCCs
→ just a few modules





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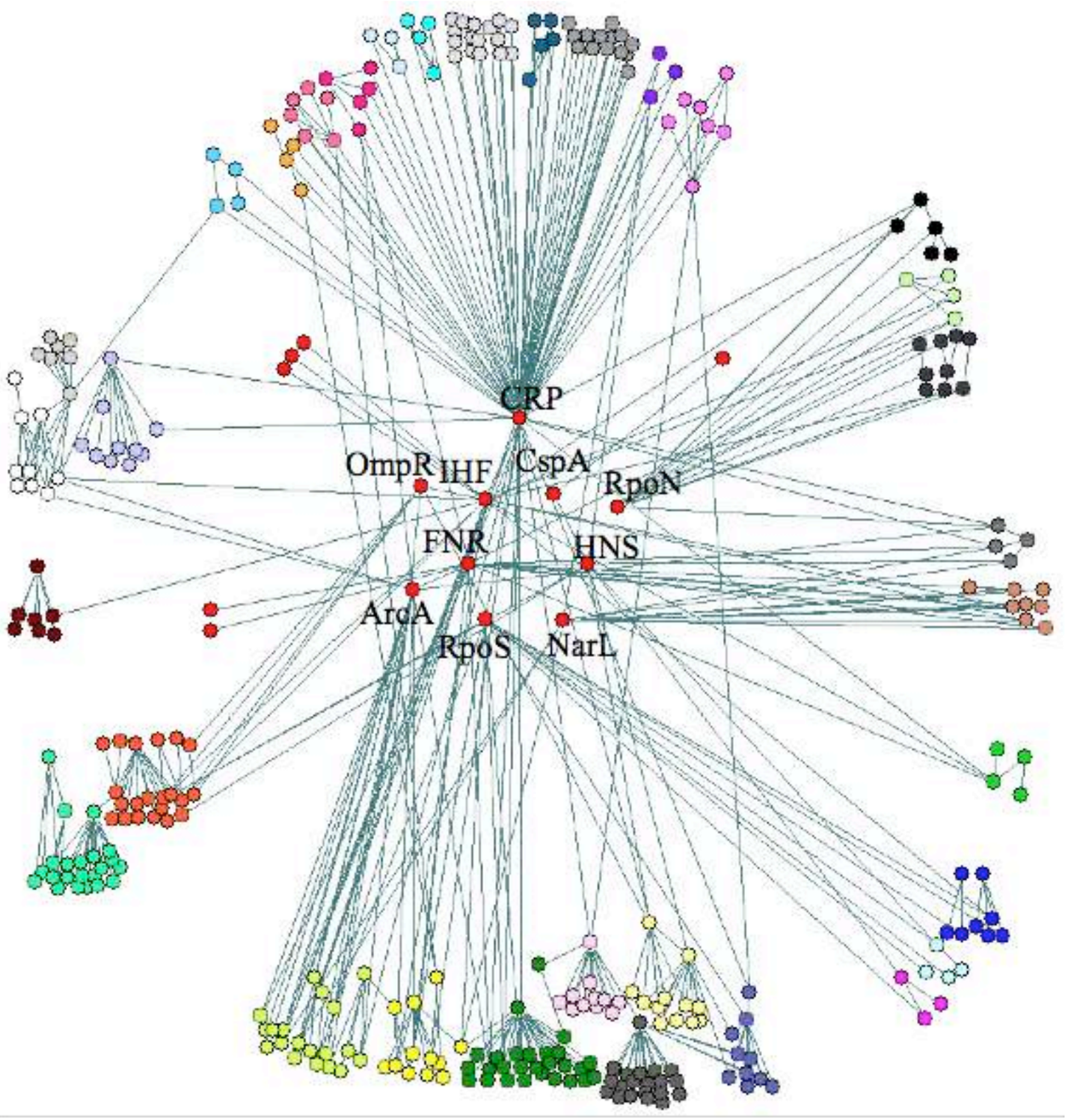
Putting it back together



The 10 global regulators are at the core of the network,

some hierarchies exist between the modules

Ma et al., *BMC Bioinformatics* **5** (2004) 199

Modules have specific functions

Table 2: Functional investigation of modules identified.

index	Operons included	Biological function description
1	*aceBAK, acs, adhE, fruBKA, fruR, icdA, iclMR, mlc, ppsA, ptsG, ptsHI_crr, pykF*	Hexose PTS transport system, PEP generation, Acetate usage, glyoxylate shunt
2	*acnA, fpr, fumC, marRAB, nfo, sodA, soxR, soxS, zwf*	Oxidative stress response
3	*ada_alkB, aidB, alkA, ahpCF, dps, gorA, katG, oxyR*	Oxidative stress response, Alkylation
4	*alaWX, aldB, argU, argW, argX_hisR_leuT_proM, aspV, dnaA, leuQPV, leuX, lysT_valT_lysW, metT_leuW_glnUW_metU_glnVX, metY_yhbC_nusA_infB, nrdAB, pdhR_aceEF_lpdA, pheU, pheV, proK, proL, proP, sdhCDAB_b0725_sucABCD, serT, serX, thrU_tyrU_glyT_thrT, thrW, tyrTV, valUXY_lysV, yhdG_fis*	rRNA, tRNA genes, DNA synthesis system, pyruvate dehydrogenase and ketoglutarate dehydrogenase system
5	*araBAD, araC, araE, araFGH, araJ*	Arabinose uptake and usage
6	*argCBH, argD, argE, argF, argI, argR, carAB*	Arginine usage, urea cycle
7	*caiF, caiTABCDE, fixABCX*	Carnitine usage
8	*clpP, dnaKJ, grpE, hflB, htpG, htpY, ibpAB, lon, mopA, mopB, rpoH*	Heat shock response
9	*codBA, cvpA_purF_ubiX, glnB, glyA, guaBA, metA, metH, metR, prsA, purC, purEK, purHD, purL, purMN, purR, pyrC, pyrD, speA, ycfC_purB, metC, metF, metJ*	Purine synthesis, purine and pyrimidine salvage pathway, methionine synthesis
10	*cpxAR, cpxP, dsbA, ecfI, htrA, motABcheAW, ppiA, skp_lpxDA_fabZ, tsr, xprB_dsbC_recJ*	Stress response, Conjugative plasmid expression, cell motility and Chemotaxis
11	*dctA, dcuB_fumB, frdABCD, yjdHG*	C4 dicarboxylate uptake
12	*edd_eda, gntKU, gntR, gntT*	Gluconate usage, ED pathway
13	*csgBA, csgDEFG, envY_ompT, evgA, gcvA, gcvR, gcvTHP, gltBDF, ilvIH, kbl_tdh, livJ, livKHMGF, lrp, lysU, ompC, ompF, oppABCDF, osmC, sdaA, serA, stpA*	Amino acid uptake and usage
14	*fdhF, fhlA, hycABCDEFGH, hypABCDE*	Formate hydrogenlyase system
15	*flgAMN, flgBCDEFGHIJ, flgKL, flgMN, flhBAE, flhDC, fliAZY, fliC, fliDST, fliE, fliFGHIJK, fliLMNOPQR, tarTapcheRBYZ*	Flagella motility system
16	*ftsQAZ, rcsAB, wza_wzb_b2060_wcaA_wcaB*	Capsule synthesis, cell division
17	*gdhA, glnALG, glnHPQ, nac, putAP*	Glutamine and proline utilization
18	*glmUS, manXYZ, nagBACD, nagE*	Glucosamine, mannose utilization
19	*glpACB, glpD, glpFK, glpR, glpTQ*	Glycerol phosphate utilization
20	*lysA, lysR, tdcABCDEFG, tdcR*	Serine, threonine usage
21	*malEFG, malK_lamB_malM, malPQ, malS, malT, malZ*	Maltose utilization

Ma et al., *BMC Bioinformatics* **5** (2004) 199

Summary

- **Static** PPI networks:
  → topology, measures, data sources, …

- **Changes** during cell cycle, adaptation to environmental changes, …
  → Gene Regulation
    → many biological steps
      → often modeled on the gene level only

Next lecture:

- Regulatory **motifs**
  → static and dynamic behavior