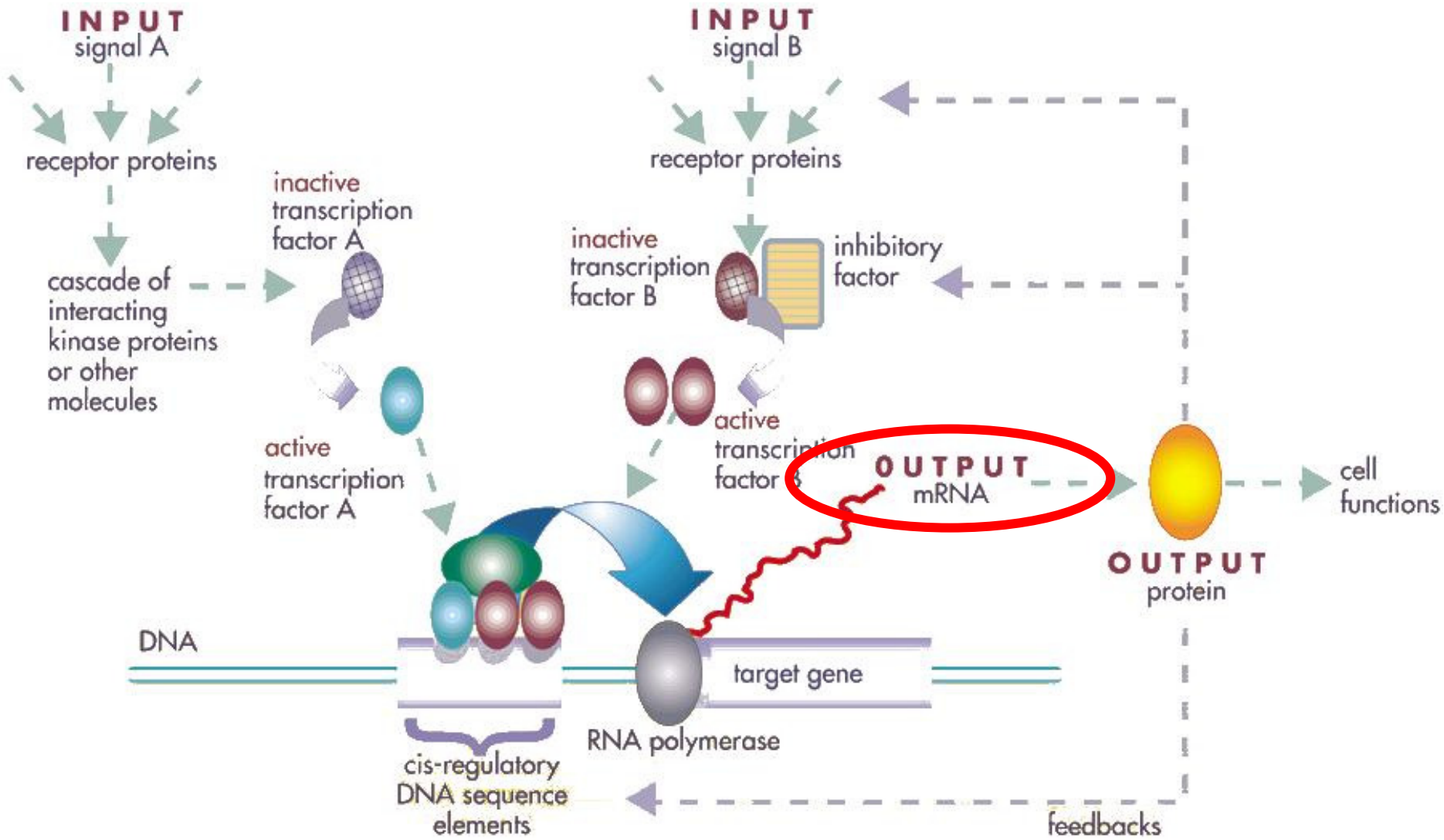
# Computational Inference of Cellular Networks from Microarray Data

Achim Tresch

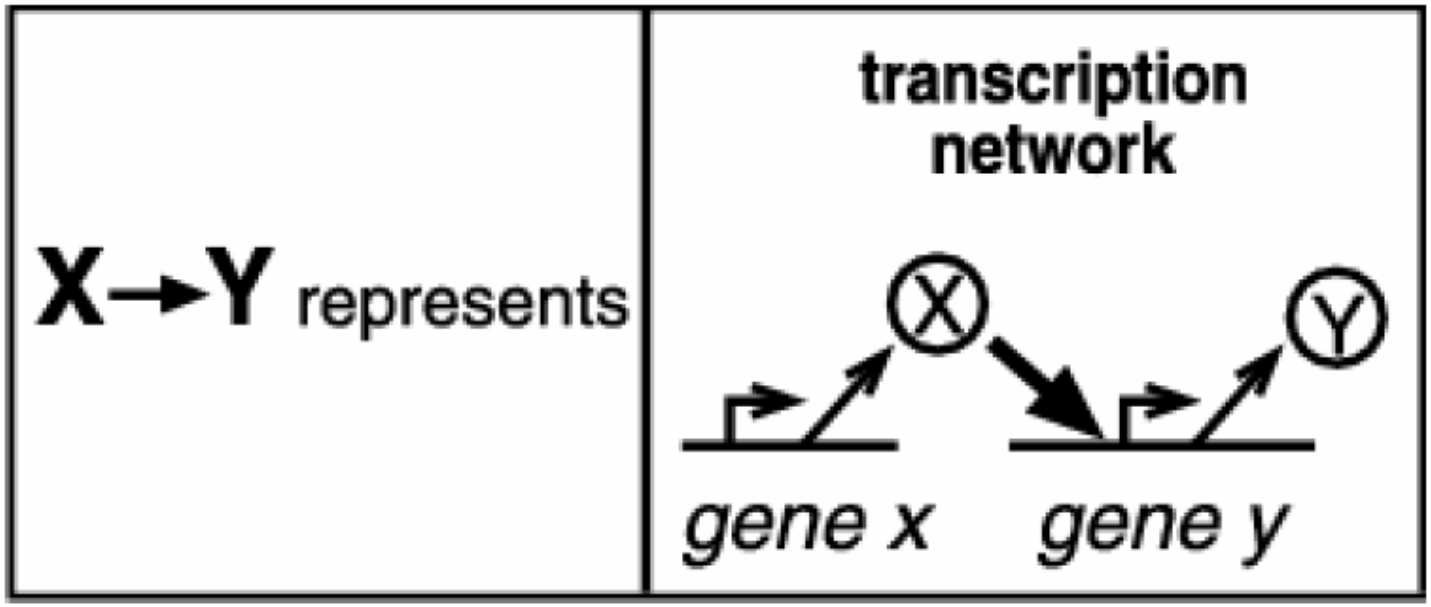
# Topics

- **Biological networks vs. Network Models**
- **Learning Networks from non-interventional data:**
  **Gaussian Graphical Models**
  **Bayesian Networks**
- **Learning from interventional data:**
  **Pruning**
  **Signal-Effects Model**

# Which biological Network?



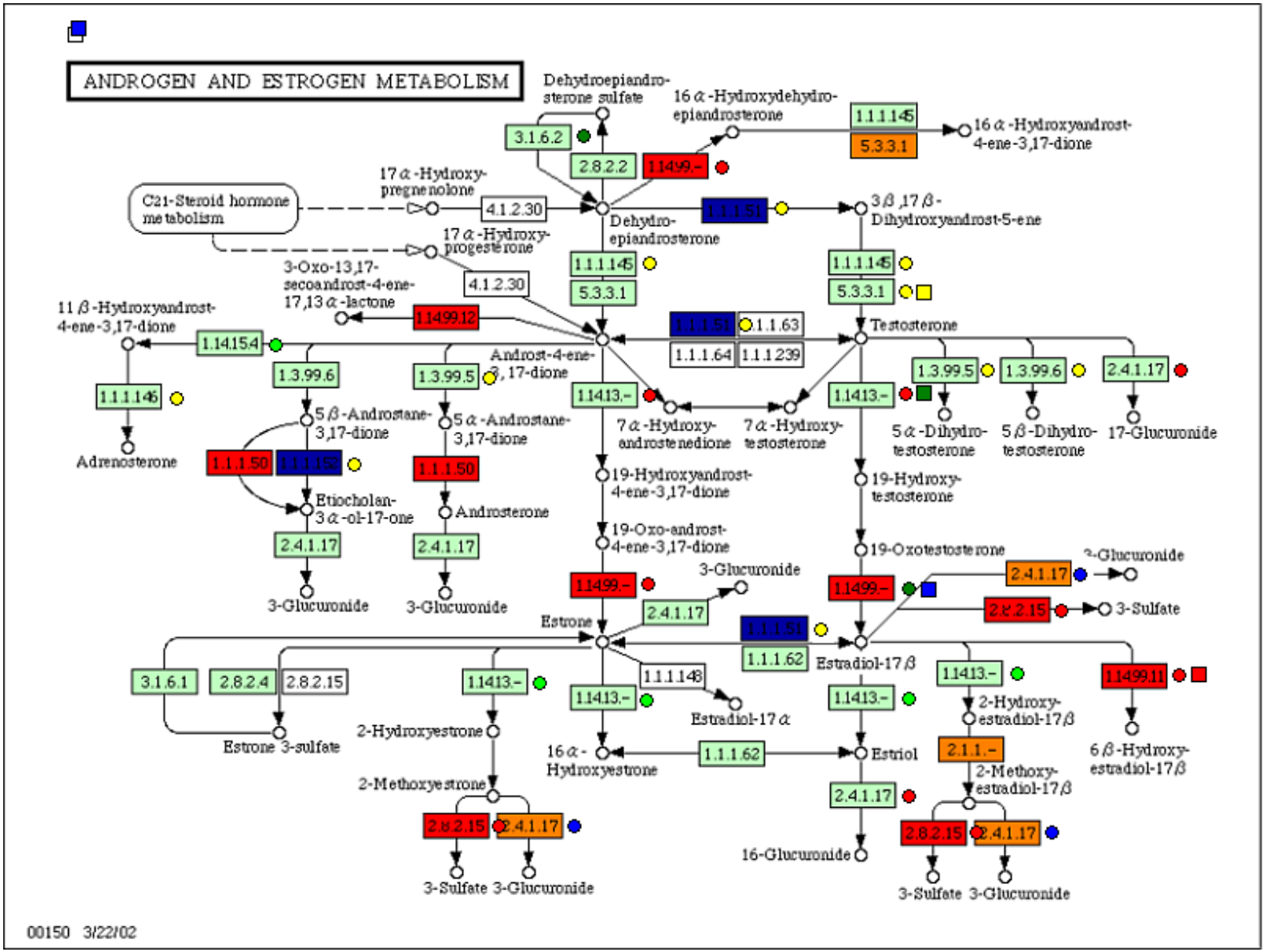
# Transcription Networks




**Nodes = transcription factors**

**Directed edge: X regulates transcription of Y**

# Transcription Networks




LEGEND

- high expression
- medium expression
- no difference
- low expression
- protein not found in array

TRANSCRIPTION FACTORS:

- Oct-1
- p107
- GATA-1
- Sp3
- Rb

DRUG TARGETS

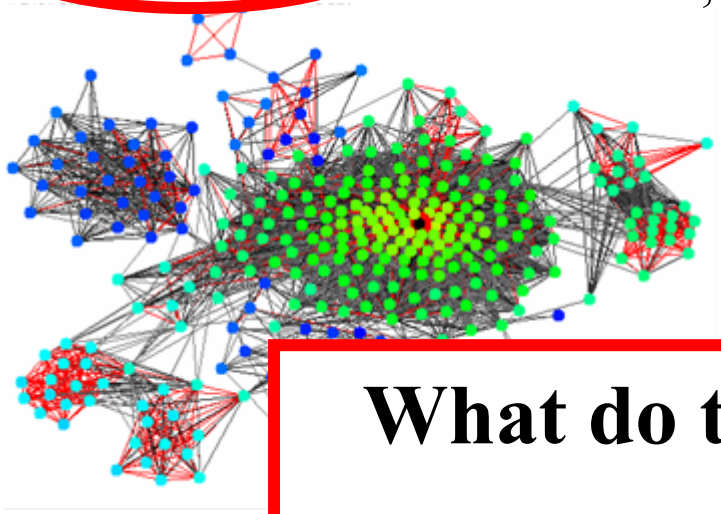
- thamoxifen
- flutamide
- anastrazole
- masterlone

From KEGG database

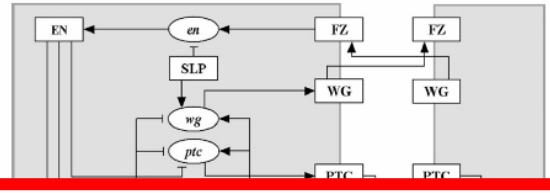
# Which Network Model?

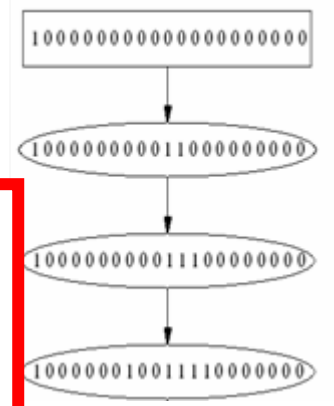
**qualitative**

Best suited for high dimensional, noisy data



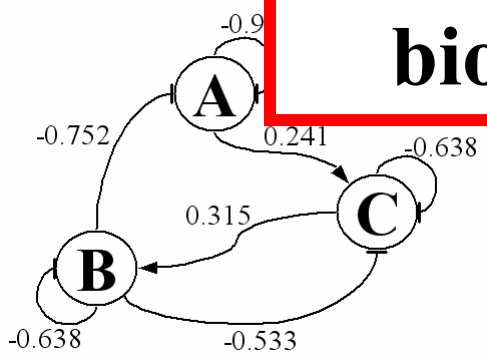
**semiquantitative**




**What do the arrows mean?**

**Can they be ascribed a biological interpretation?**




$$\frac{d[B]}{dt} = \frac{V_b}{1+\frac{K_{aC}}{[C]}} - k_b[B]$$

$$\frac{d[C]}{dt} = \frac{V_c}{\left(1+\frac{[B]}{K_{iB'}}\right)\left(1+\frac{K_{aA}}{[A]}\right)} - k_c[C]$$

**quantitative**

# Qulitative Network Models

**Possible Models include**

- **Correlation Graphs**
- **Gaussian Graphical Models**
- **Bayesian Networks**

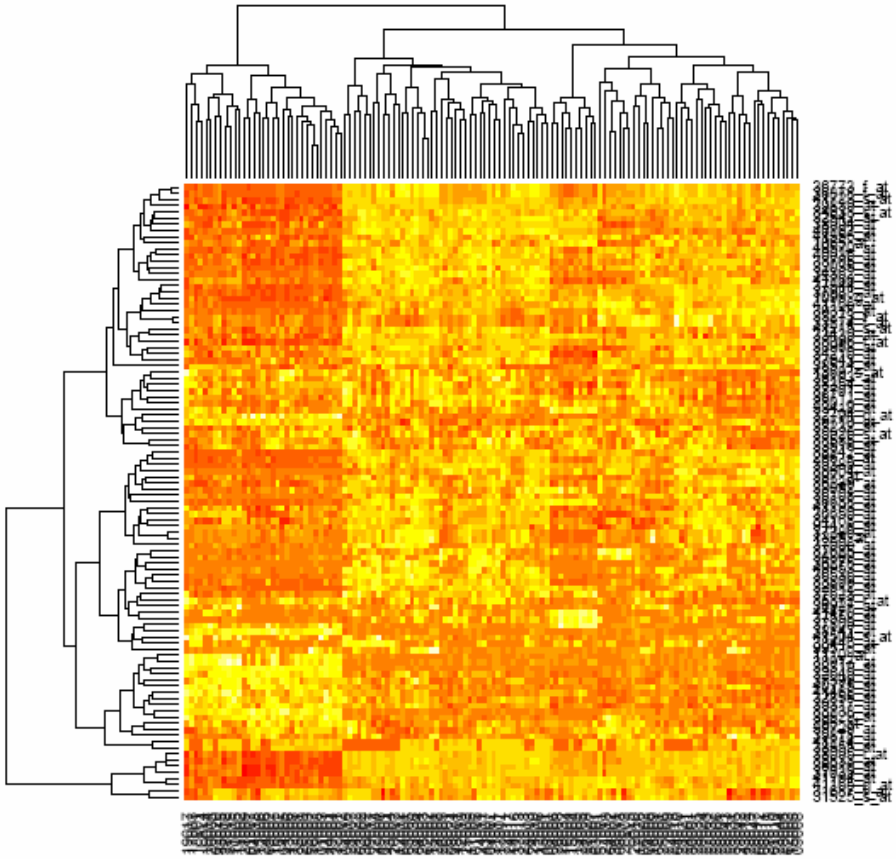
**However: Correct Reconstruction of the complete regulatory network is impossible due to**

- **Lack of data**
- **Measurement error**
- **Oversimple/wrong model assumptions**

**“All models are wrong, some of them are useful“**

**(Edwards Deming, George Box)**

# Clustering by coexpression



Assumption:

Coexpression $\sim$ coregulation

If genes show the same expression profiles they follow the same regulatory regimes

# Correlation Graphs

- An expression profile is a collection of expression vectors
$$\{ X_g = (X_{g,s})_{s \in \text{samples}} , g \in \text{Genes} \}$$

- Correlation graph: Depict genes as vertices of a graph and draw an undirected edge (i, j) if some correlation measure (Pearson correlation, Spearman rank correlation, Kendall's tau) between $X_i$ and $X_j$ is sufficiently different from zero.

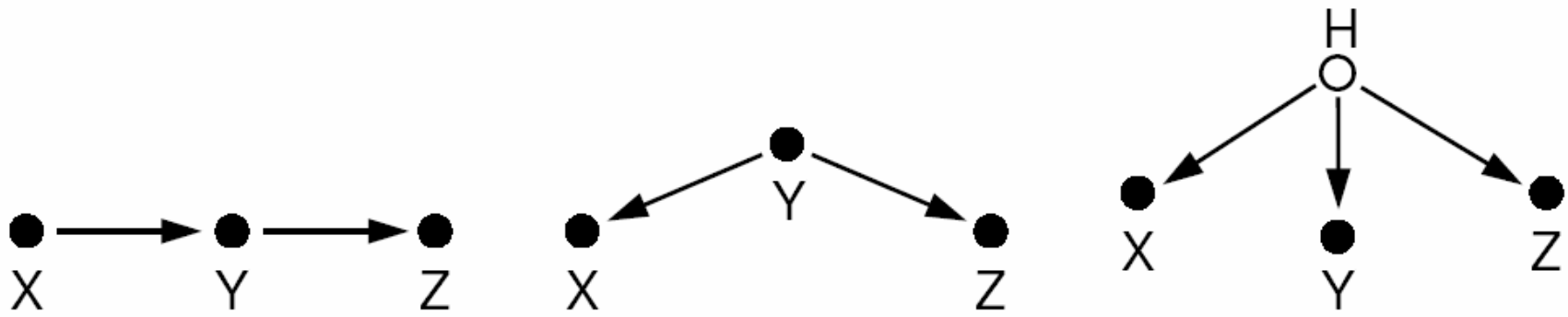
- Advantage: This representation of the marginal dependence structure is easy to interpret and can be accurately estimated even if p » N.

- Application: Stuart et al (Science, 2003) build a graph from coexpression across multiple organisms.

# Problems of correlation based approaches

- **It is impossible to distinguish direct from indirect dependencies**

  **Three reasons why X, Y , and Z may be highly correlated:**




**Possible remedies:**

- **search for correlations which cannot be explained by other variables.**
- **measure effects of gene perturbations**

- **A strong correlation is not a strong evidence for regulatory dependence (lots of false positives) rather than a low correlation is a strong evidence for no regulatory edge.**

# Conditional Independence

Be $X, Y, Z$ random variables with joint distribution $P$.

**$X$ is conditionally independent of $Y$ given $Z$**

$$X \perp\!\!\!\perp Y \mid Z \quad \Leftrightarrow$$

$$P(X = x, Y = y | Z = z) = P(X = x | Z = z) \cdot P(Y = y | Z = z)$$
$$P(X = x | Y = y, Z = z) = P(X = x | Z = z)$$

**In other words:**

- **Knowing Z, knowing Y is irrelevant for knowing X (and vice versa).**
- **Z explains any observed dependency between X and Y .**

# Gaussian Graphical Models (GGM)

Given a random vector $\mathbf{X} = (X_1, \ldots, X_p)$.

A Gaussian graphical model [7, 4] is an **undirected graph** on vertex set $V$, with $|V| = p$ .

To each vertex $i \in V$ corresponds a **random variable** $X_i \in \mathbf{X}$.

Draw an **edge** between vertices $i$ and $j$ if and only if

$$X_i \not\perp\!\!\!\perp X_j \mid \mathbf{X}_{\text{rest}},$$

where $\mathbf{X}_{\text{rest}} = \mathbf{X} \backslash \{X_i, X_j\}$.

# Gaussian Graphical Models (GGM)

**If we assume that the common expression distribution of all genes follows a multivariate Gaussian distribution (which is of course never the case), conditional independence can be assessed as follows:**

1. First estimate the covariance matrix $\Sigma$ by the sample covariance matrix

$$\hat{\Sigma} = \frac{1}{N-1}(X - \bar{X})^T(X - \bar{X}).$$

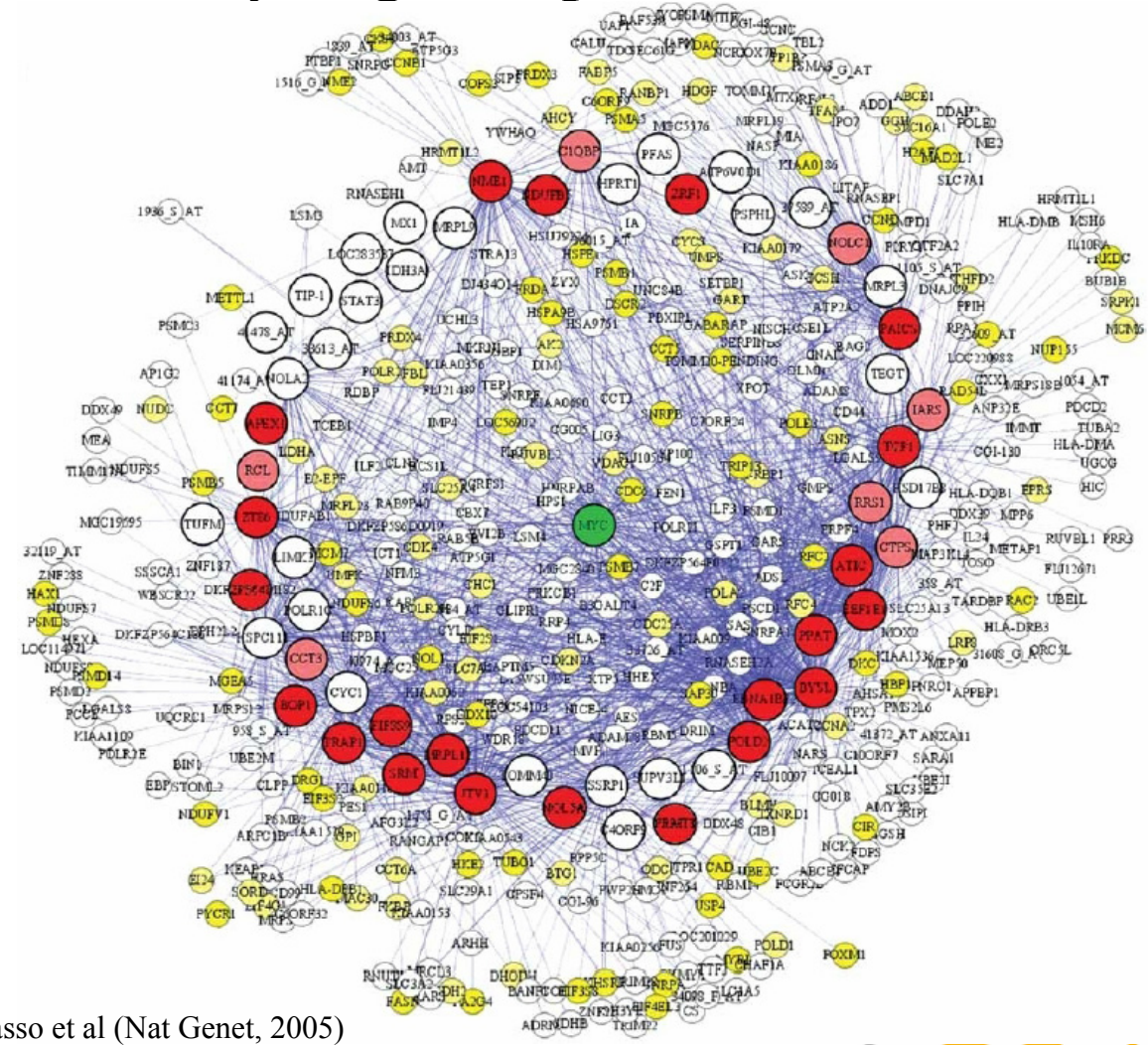
2. Invert $\hat{\Sigma}$ to obtain an estimate $\hat{K}$ of the precision matrix $K$.
3. Employ statistical tests [56] to decide, which entries in $\hat{K}$ are significantly different from zero.

# What if p » N?

**Full conditional relationships can only be accurately estimated if the number of samples N is relatively large compared to the number of variables p.**

**Thus, if p » N, you can . . .**

- **use the Moore-Penrose pseudoinverse and bootstrap aggregation and shrinkage estimators to stabilize the result (e.g. Schäfer and Strimmer, Bioinformatics´05)**
- **resort to a simpler model that does not rely on full conditional independence**



Graph from Basso et al (Nat Genet, 2005)

# Modified Gaussian Graphical Models (GGM)

We have seen methods to build graphs from

1. marginal dependencies

$$X_i \not\perp\!\!\!\perp X_j \mid \emptyset$$ Correlation Graphs

2. full conditional dependence

$$X_i \not\perp\!\!\!\perp X_j \mid X_{\text{rest}}$$ GGMs

3. first order dependencies

$$X_i \not\perp\!\!\!\perp X_j \mid X_k \quad \forall k \in \text{rest}$$ Wille / Bühlmann

4. This leads use to include **all higher order dependencies**

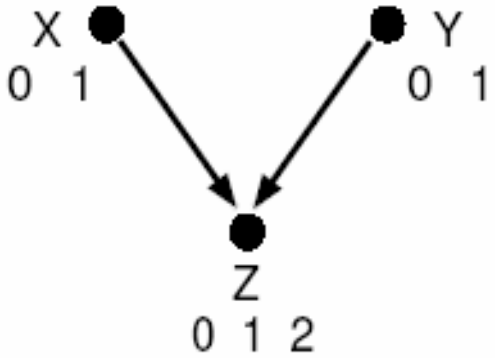
$$X_i \not\perp\!\!\!\perp X_j \mid \mathbf{X}_S \quad \text{for all } S \subseteq \text{rest}$$

**All methods failed to accurately reconstruct networks, even if they were of very moderate size (~20)**

# Children depend on parents

The DAG defines families.

Relationships are further characterized by local probability distributions:




$$p(x) = (0.6 \quad 0.4)$$

$$p(y) = (0.2 \quad 0.8)$$

$$p(z|x,y) = \begin{cases} (0.8 \quad 0.1 \quad 0.1) & \text{if } (X,Y) = (0,0) \\ (0.1 \quad 0.8 \quad 0.1) & \text{if } (X,Y) = (0,1) \\ (0.1 \quad 0.8 \quad 0.1) & \text{if } (X,Y) = (1,0) \\ (0.1 \quad 0.1 \quad 0.8) & \text{if } (X,Y) = (1,1) \end{cases}$$

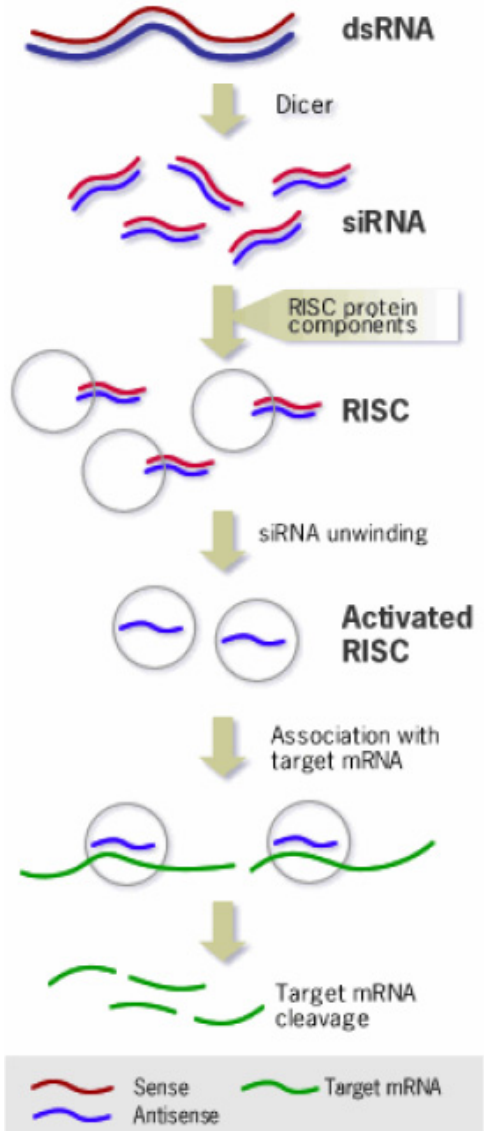
# Bayesian Networks

**Problems:**

- **Given a directed acyclic graph (DAG), learn the local probability distributions and score the DAG according to its likelihood („how good does this graph fit the data“?)**
- **Find the topology(-ies) for the underlying DAG**

**The latter point is the crucial problem, since there may be DAGs that are equally likely, and there are in general millions of DAGs that score comparably well.**

**Besides that, a directed edge does not necessarily imply a causal relation between the adjacent nodes.**

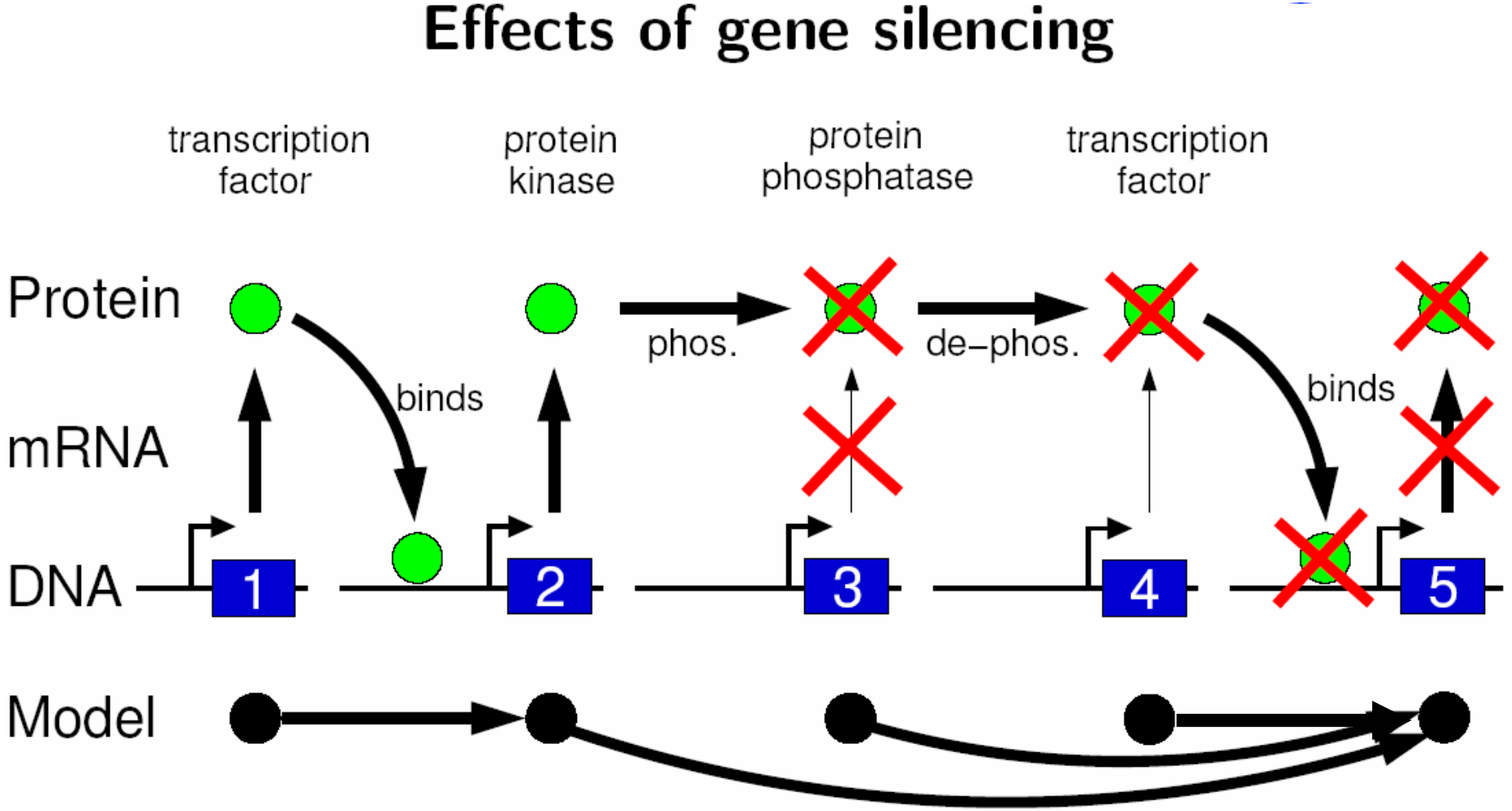



# RNAi kills the messenger

1. **Double-stranded RNAs** (dsRNAs) get processed into **small interfering RNAs** (siRNAs).
2. siRNAs assemble into **RNA-induced silencing complexes** (RISCs).
3. The siRNA strands guide the RISCs to **complementary RNA molecules**, . . .
4. . . . where they **cleave and destroy** the cognate RNA.

Figure from http://www.ambion.com

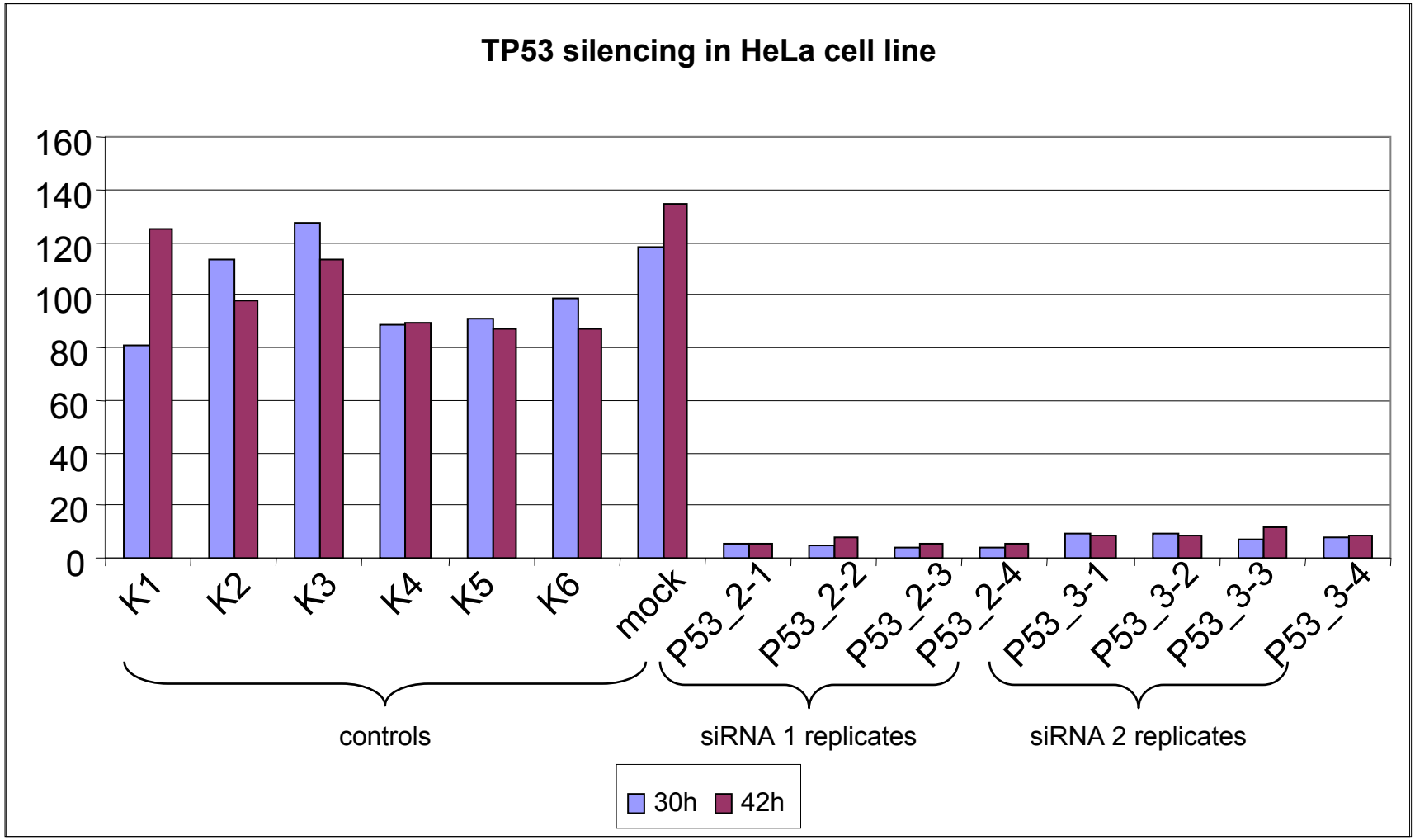
# Learning from interventional data



# Proof-of-principle Experiment

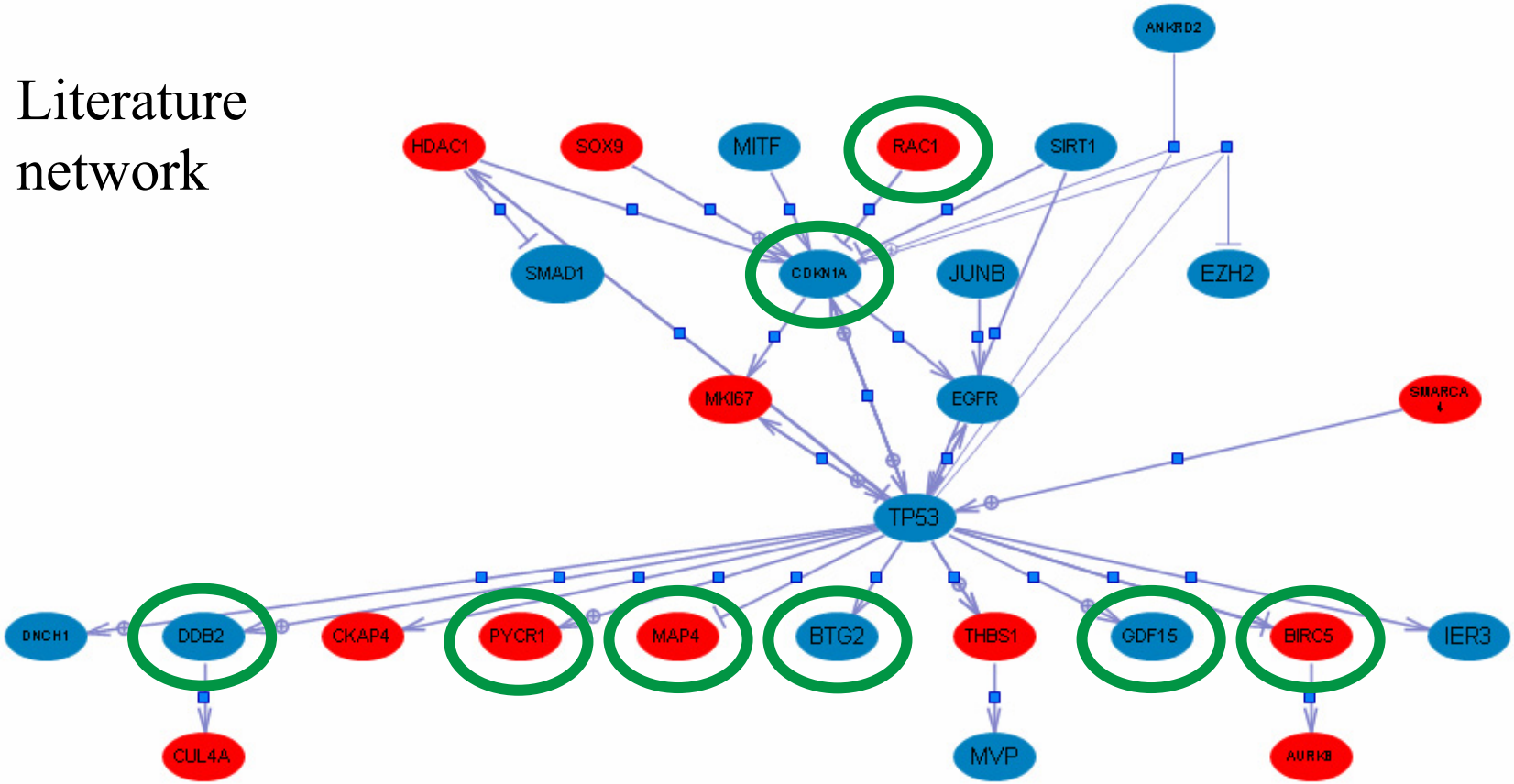
- TP53 chosen as model-gene for siRNA mediated knock-down
- nuclear protein, DNA-binding, postulated to bind as tetramer, activates expression of downstream genes that inhibit growth and/or invasion, thus function as a tumor suppressor
- very well known in literature, ease to find interaction partners
- Transfection of siRNA into HeLa cell line using HiPerFect transfection-reagent (HeLa cell line known to show very good transfection efficiencies)
- Minimal concentration of siRNA used to reduce possible off-target effects
- Measurement of silencing efficiency regarding mRNA of TP53 using qRT-PCR

# Proof-of-principle Experiment



# Proof of principle: The TP53 network

Literature network




increase

decrease

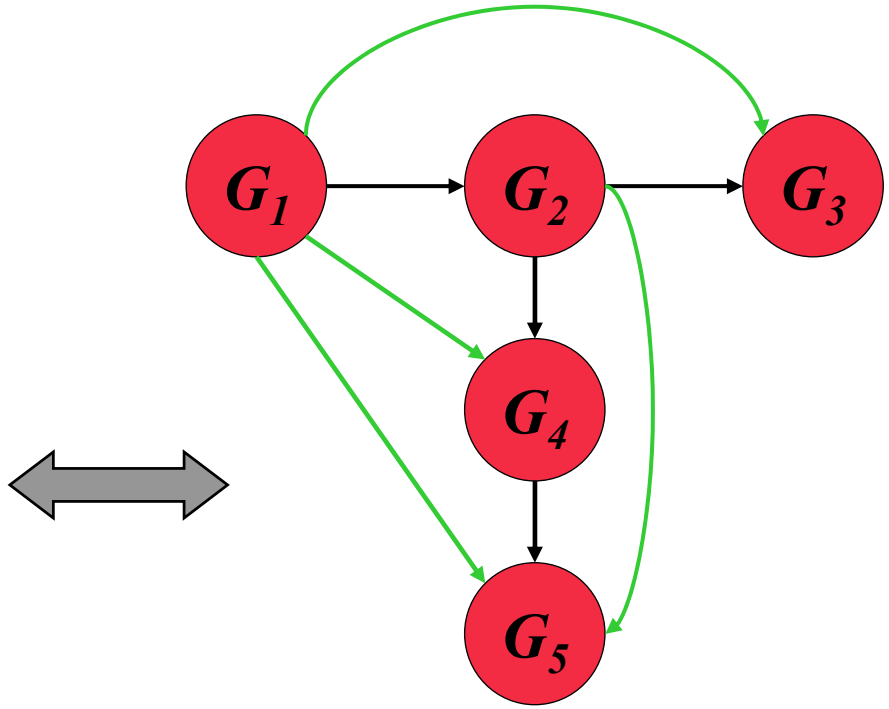
found by siRNA experiment

# Pruning of Gene interaction Graphs

**observations list**

| Pertur-bation | Effect |
|---|---|
| $G_1$ | $G_2$ , $G_3$ , $G_4$ , $G_5$ |
| $G_2$ | $G_3$ , $G_4$ , $G_5$ |
| $G_3$ | - |
| $G_4$ | $G_5$ |
| $G_5$ | - |

**Interaction graph**




necessarily direct interactions

optional, possibly indirect interactions

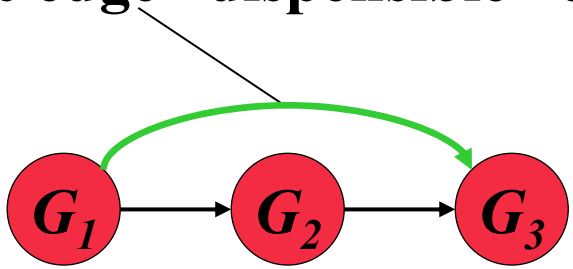
# Pruning of Gene interaction Graphs

**Given a gene interaction graph, find edges that survive Occam's razor (14th century):**

"***non est ponenda pluritas sine necessitate***"

*(pluralities ought not to be proposed without necessity)*

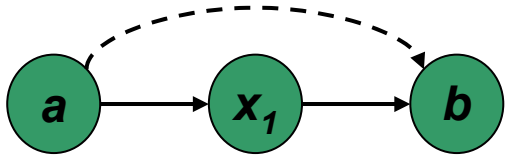
**Is this edge "dispensible" or not?**



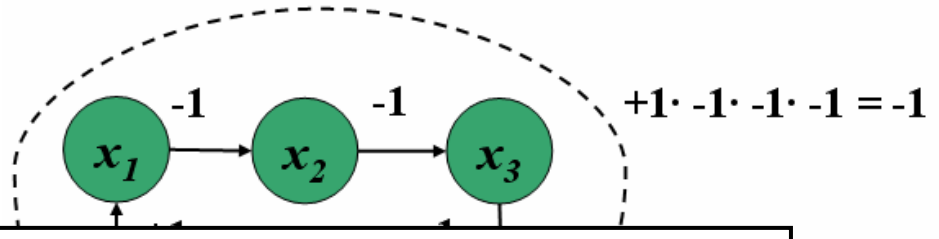

**Need for algorithm to define and find**
**minimal consistent and biologically meaningful graph**

# Finding non-necessary edges

- "Trivial".
  Remove all edges a→b for which there exists a bypass (a longer way from a to b). [Wagner, 2002]

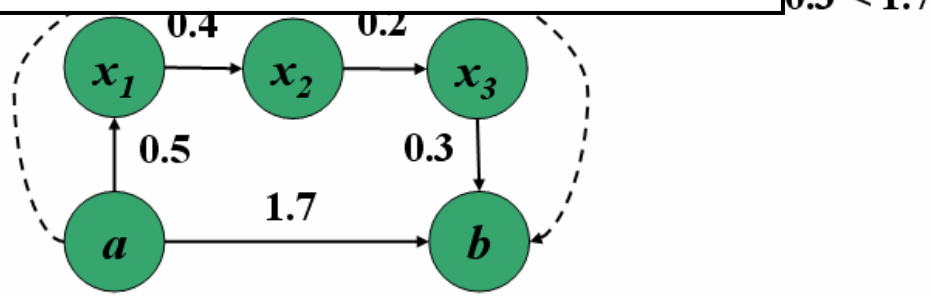



- "Signs".
  Let every edge of the observational graph hava a sign +1 or –1 according to the d
  Rem
  along
  of the




**The methods do not remove enough edges, too many false positives.**
**Too many interventions (and replicate measurements of them) needed for reliable estimation.**

- "Wei
  Let e
  negative number. Edges with low weights are meant to represent edges for which there is strong evidence for a direct regulatory interaction.
  Remove a→b if sum of the weights along the path a→... →b is smaller than the weight of the edge a→b
  [Tresch et al.]




*Tresch et al, unpublished*

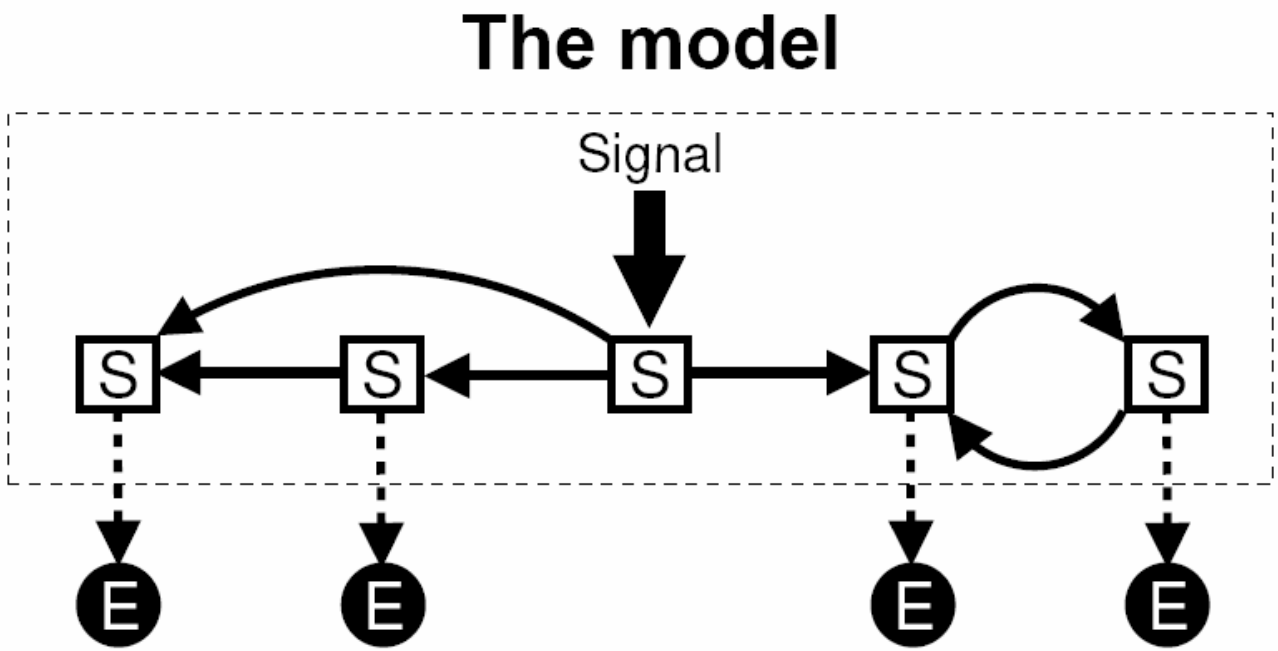
# Experiments and Data

Do microarrays for:

1. **Negative controls** — no signal, no interventions
2. **Positive controls** — pathway activated by signal, no interventions
3. **Interventions** — while signal is on!

**Data:** binary matrix $D = (e_{ik})$,
where $e_{ik} = 1$ if E-gene $E_i$ shows in experiment $k$ the same expression as in the negative controls.

# Signal-Effects Model




A pathway is an **unrestricted directed graph** with S-genes as nodes.

Each E-genes is attached to a single S-gene. It reacts to interventions at all S-genes which are upstream in the pathway.

# Signal-Effects Model

- **Distinguish between:**
  - **S-genes (for "signaling" or "silenced"): pathway genes.**
  - **E-genes (for "effects"): reporters for S-gene activity.**
- **S-genes do not show expression changes when other S-genes are silenced (*i.e.* no overlap between S-genes and E-genes)**
- **Pathway has to be constructed from secondary effects at E-genes (= transcriptional phenotypes)**
- **Procedure:**
  - **Consider a given candidate pathway topology on S-genes.**
  - **Step 1. Assume that positions of E-genes are known. Calculate the likelihood of the data.**
  - **Step 2. Reality: positions of E-genes are unknown! Average likelihoods for all possible positions of E-genes**
  - **Step 3. Given the most likely topology, Estimate the most probable position of E-genes.**

# Pathways from RNAi data – an example


## Response to microbial challenge

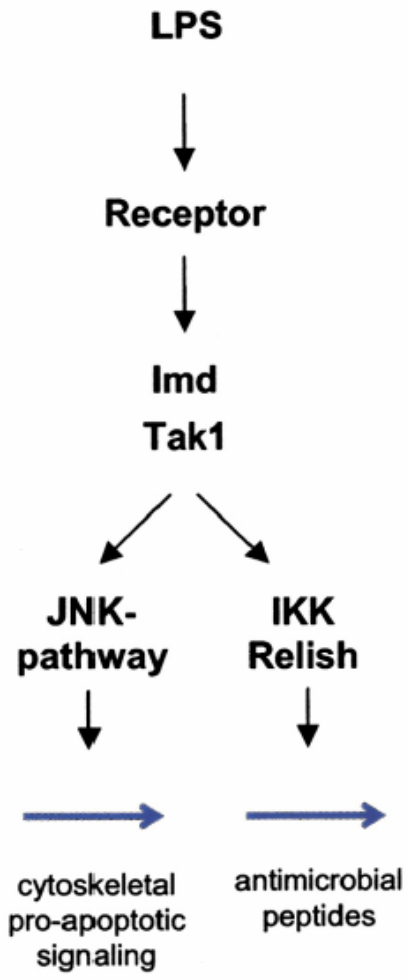
(Boutros *et al.*, Dev Cell, 2002)

Columns: silenced genes.

Rows: effects on other genes.

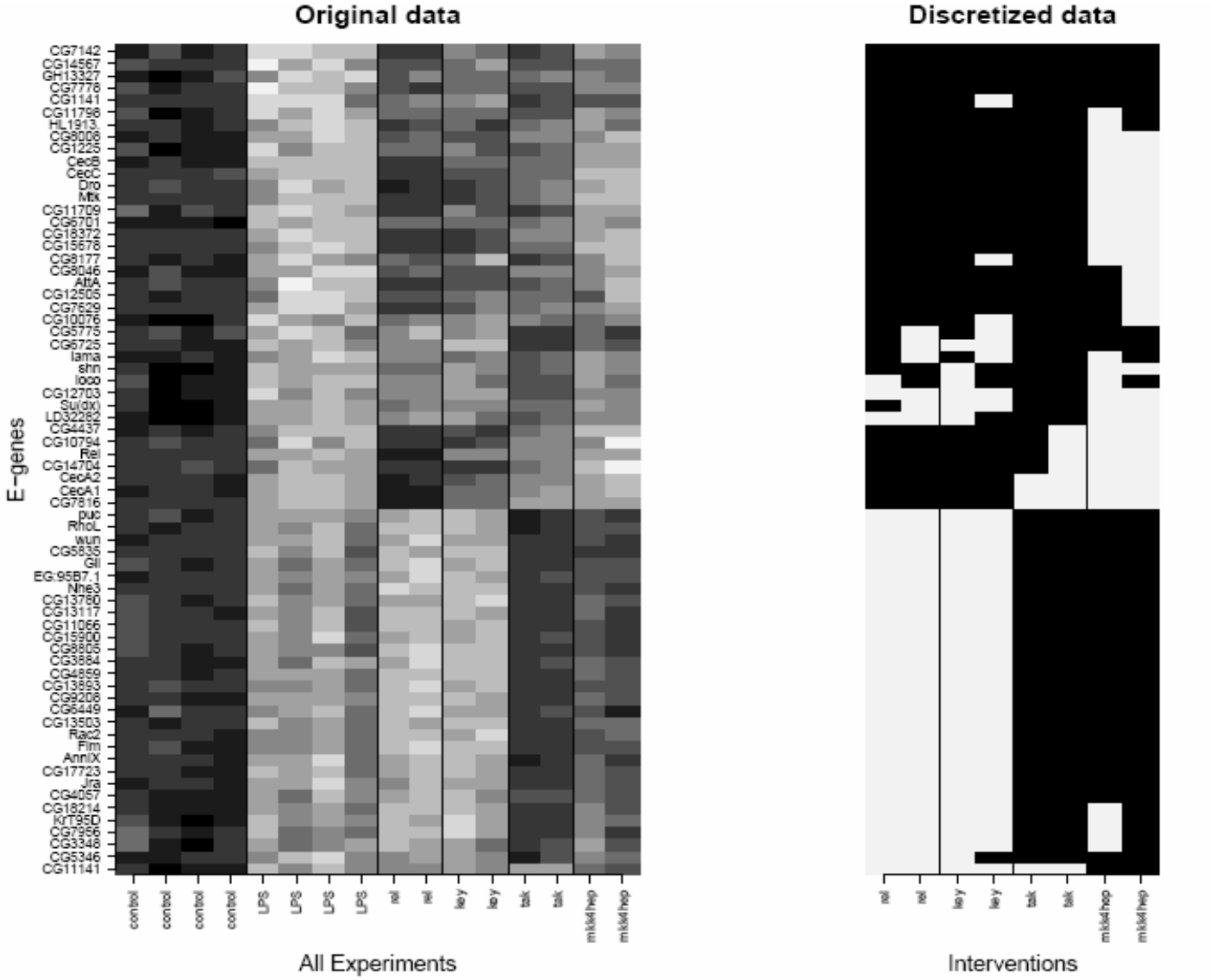
## Results:

1. Silencing tak1 reduces expression of all LPS-inducible transcripts.
2. Silencing rel (key) or mkk4/hep reduces expression of separate sets of induced transcripts.

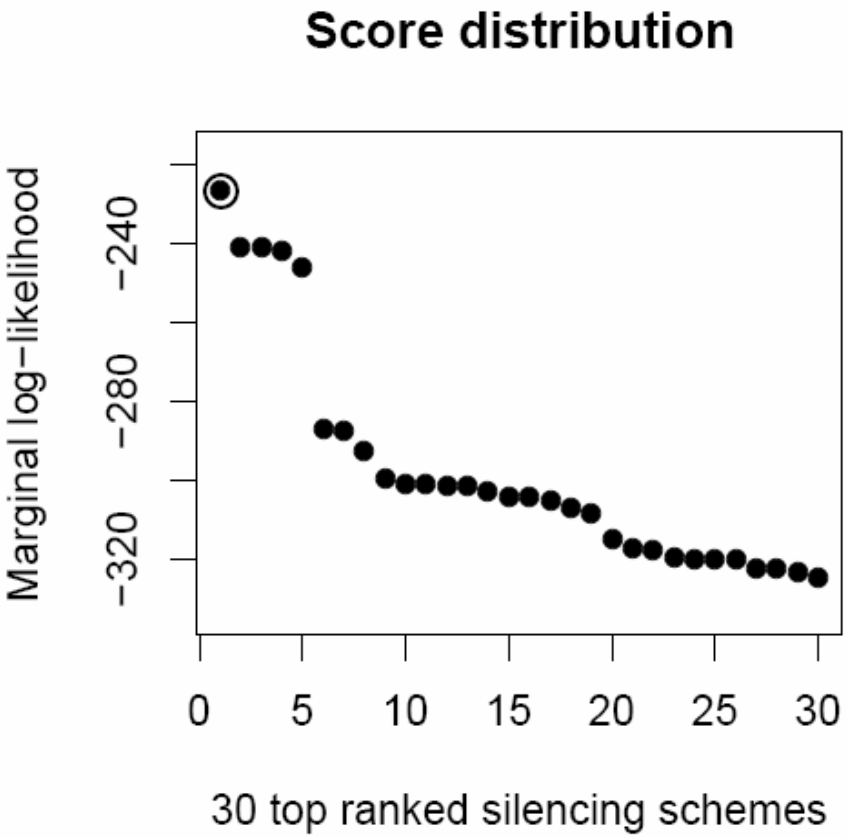


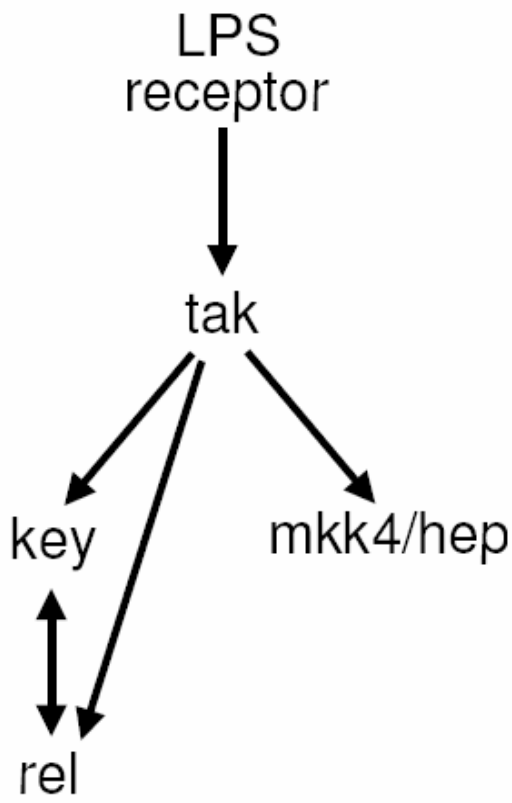

Figures from (Boutros *et al.*, 2002)

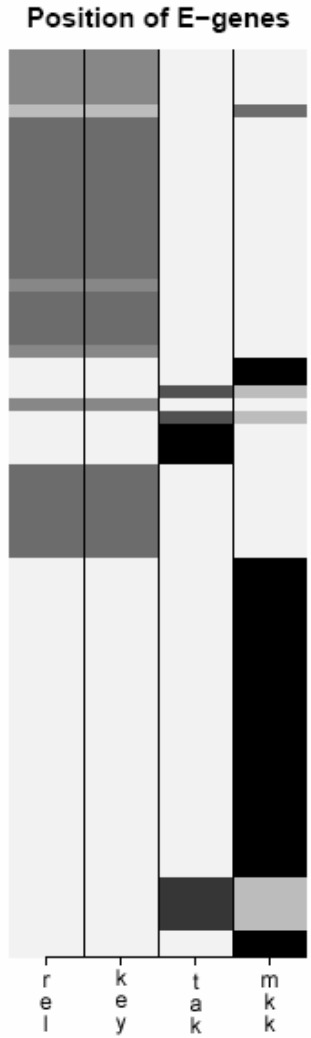
# Application to Drosophila data



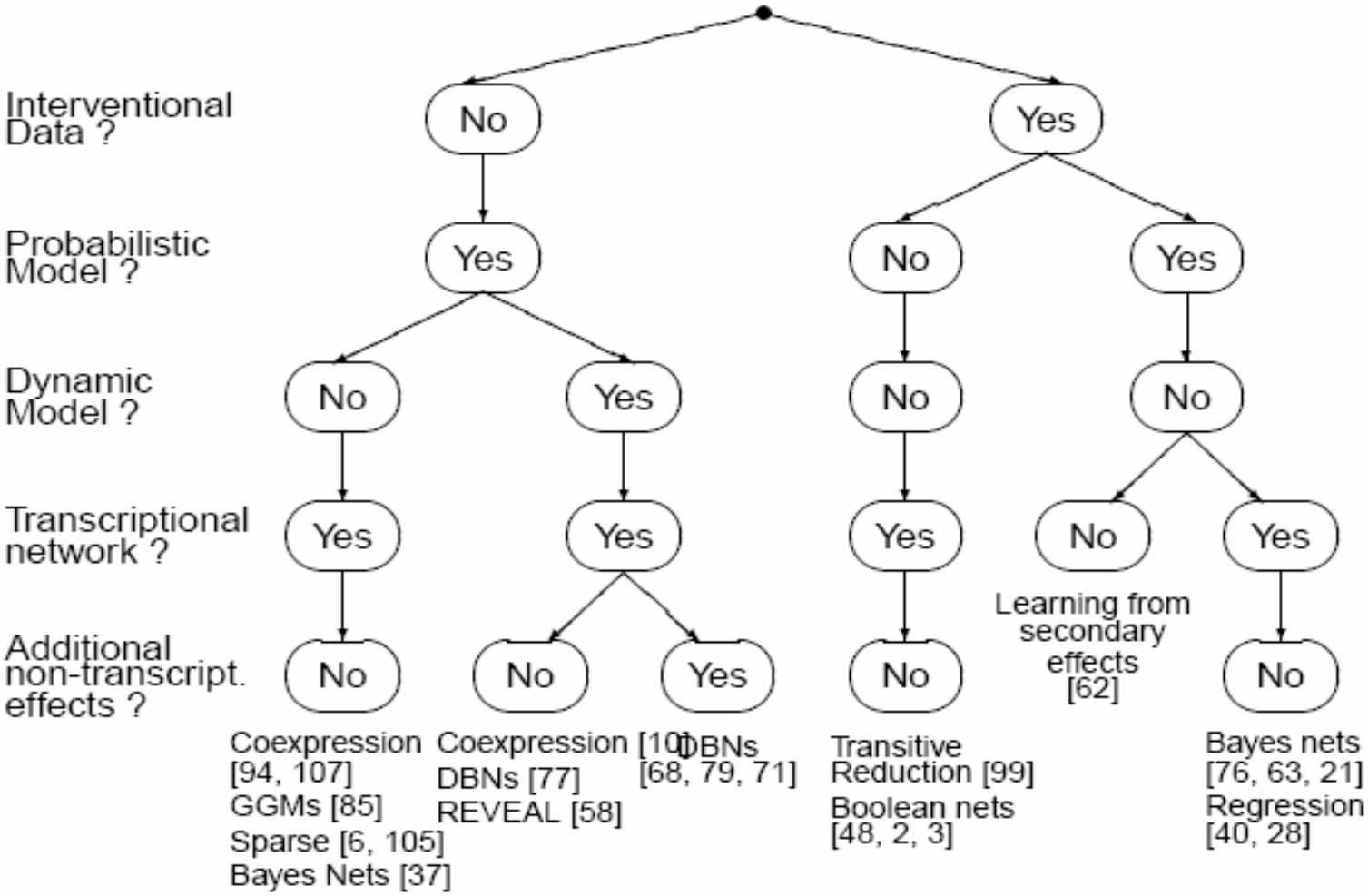
# Application to Drosophila data



# Survey of network models




Florian Markowetz, *Computational Inference of Cellular Pathways*, 2005 Oct 5

# Networks in R/Bioconductor

- graph: basic class definitions and functionality
- RBGL: interface to graph algorithms (e.g. shortest path, connectivity)
- Rgraphviz: rendering functionality Different layout algorithms. Node plotting, line type, colour etc. can be controlled by the user.
- dynamicGraph: visualize interactive Graphs with TclTK.
- KEGGSOAP: Connecting to the KEGG database.
- GeneTS: Estimate GGMs from Microarray Data.

# Pathways and Visualization Tools

- **Some Pathway Databases:**
  - **KEGG (http://www.genome.jp/kegg)**
  - **TRANSPATH (http://www.biobase.de)**
  - **Biocarta (http://www.biocarta.com)**
  - **Reactome (http://www.reactome.org)**
  - **HumanCyc (http://humancyc.org)**
  - **Signal Transduktion Knowledge Environment (http://stke.sciencemag.org)**

- **Software tools**
  - **GeneMAPP (www.genemapp.org)**
  - **GoMiner (http://discover.nci.nih.gov/gominer)**
  - **Bioconductor/Graphviz (http://www.bioconductor.org)**
  - **Cytoscape (http://www.cytoscape.org)**

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