**Modeling of Six Related, Database-Derived GRNs (Slides 2-71)**

Six database-derived small gene regulatory networks (GRNs) with between 14-17 genes and 25-35 edges were modeled using GRNmap. Two separate networks (db2 and db3) were derived from the ∆cin5 strain data, as the removal of the transcription factor MCM1 from the larger 17-gene network (db3) further disconnected ACE2 and ZAP1 from the network. The naming scheme for the six networks is as follows:

* **db1: network derived from wild-type data**
  + 16 genes, 26 edges
* **db2: network derived from ∆cin5 data**
  + 14 genes, 25 edges
* **db3: network derived from ∆cin5 data**
  + 17 genes, 32 edges
* **db4: network derived from ∆gln3 data**
  + 14 genes, 35 edges
* **db5: network derived from ∆hap4 data\***
  + 15 genes, 28 edges
* **db6: network derived from ∆zap1 data**
  + 16 genes, 27 edges

Note: db5, marked with an asterisk (\*) above, was used as model network for comparison to randomly generated, small GRNs. It exhibited the lowest theoretic minimum LSE of the six database-derived networks, indicating that it was best able to model the transcriptional responses observed in *Saccharomyces cerevisiae* during cold shock.

**I. Comparative Analysis of the Six Database-Derived GRNs**

**Table 1.** GRNmap network parameter outputs for db1-db6.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Parameter** | **db1** | **db2** | **db3** | **db4** | **db5** | **db6** |
| Penalty Term | 2.5923 | 1.8830 | 1.7570 | 1.7895 | 2.3443 | 1.8276 |
| LSE | 0.8194 | 0.6634 | 0.7864 | 0.6994 | 0.6919 | 0.8602 |
| minLSE | 0.5768 | 0.4885 | 0.5449 | 0.5379 | 0.4851 | 0.6156 |
| LSE:minLSE Ratio | 1.4206 | 1.3580 | 1.4100 | 1.3000 | 1.4263 | 1.3973 |
| Iteration Count | 109,718 | 53,862 | 118,921 | 78,124 | 62,139 | 76,769 |

* LSE:minLSE ratios for db1-db6 ranged from 1.3000 (db4) to 1.4263 (db5), indicating that GRNmap consistently modeled the dynamics of small GRNs well.
* The database-derived network with the lowest theoretical minimum LSE was db5 (0.4851), indicating that this network best accounted for the regulatory dynamics observed in *Saccharomyces cerevisiae* during cold shock.
  + db5 contains an intermediate number of genes and edges (15, 28).
* Conversely, db6 exhibited the highest theoretical minimum LSE (0.6156), suggesting that this network is least likely to account for the transcriptional changes observed in *S. cerevisiae* during cold shock.
  + db6 contained several different transcription factors compared to db1-db5. Specifically, db6 was the only network to include CST6 and HSF1, whereas it was also the only network to exclude SFP1, YHP1, and YOX1 (Table 2).

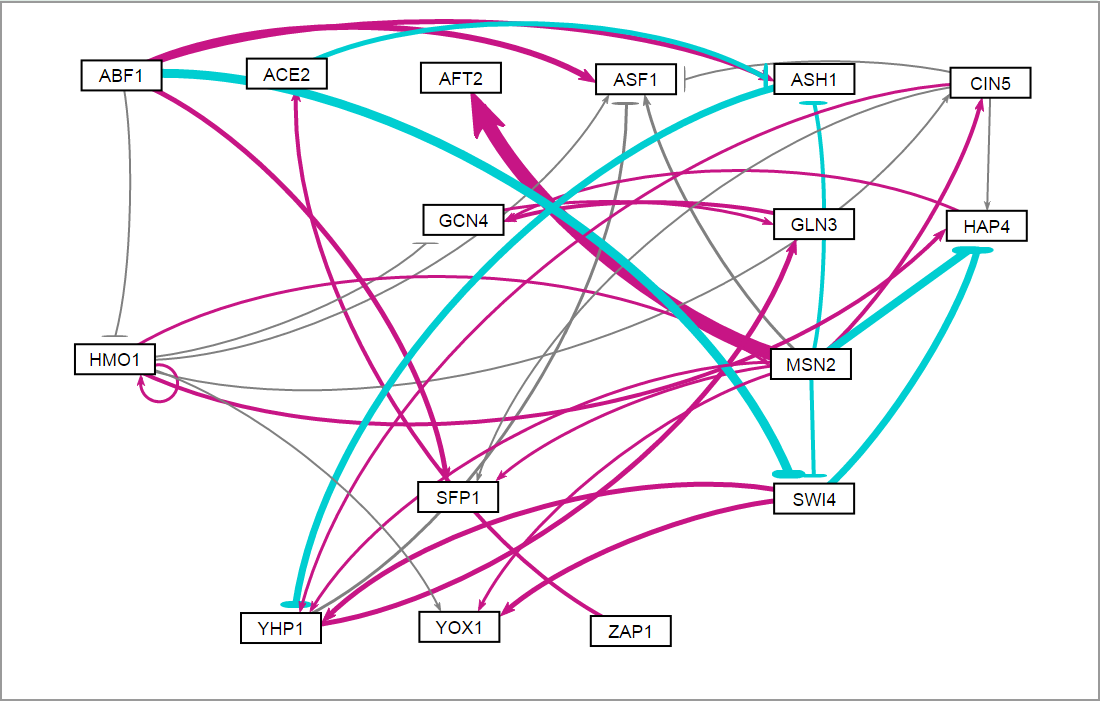
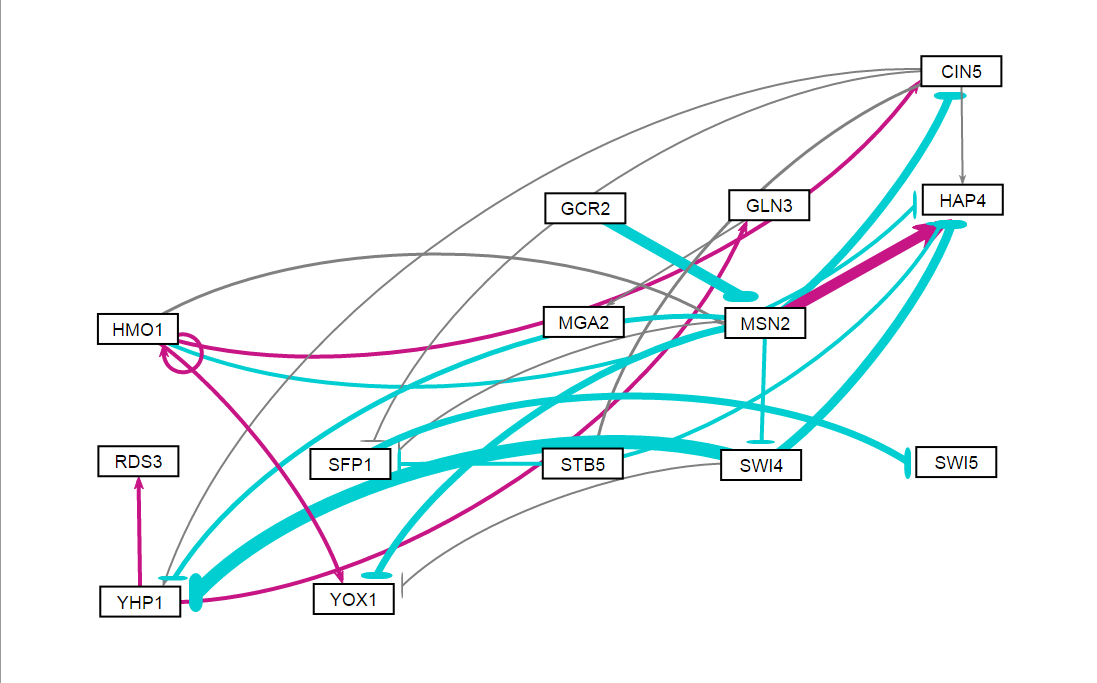
**Table 2.** Gene list for db1-db6. Factors that were deleted in tested strains of *Saccharomyces cerevisiae* are highlighted in yellow. Counts of the number of networks in which a gene is represented are displayed on the right.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **All Genes** | **db1** | **db2** | **db3** | **db4** | **db5** | **db6** | **Count** |
| ABF1 | ABF1 |  |  |  |  | ABF1 | 2 |
| ACE2 | ACE2 |  | ACE2 |  | ACE2 | ACE2 | 4 |
| AFT2 | AFT2 |  |  |  |  |  | 1 |
| ASF1 | ASF1 |  |  |  |  |  | 1 |
| ASH1 | ASH1 |  |  |  | ASH1 |  | 2 |
| CIN5 | CIN5 | CIN5 | CIN5 | CIN5 | CIN5 | CIN5 | 6 |
| CST6 |  |  |  |  |  | CST6 | 1 |
| CYC8 |  |  |  | CYC8 |  |  | 1 |
| GCN4 | GCN4 |  |  |  |  | GCN4 | 2 |
| GCR2 |  | GCR2 | GCR2 | GCR2 | GCR2 | GCR2 | 5 |
| GLN3 | GLN3 | GLN3 | GLN3 | GLN3 | GLN3 | GLN3 | 6 |
| HAP4 | HAP4 | HAP4 | HAP4 | HAP4 | HAP4 | HAP4 | 6 |
| HMO1 | HMO1 | HMO1 | HMO1 | HMO1 | HMO1 | HMO1 | 6 |
| HSF1 |  |  |  |  |  | HSF1 | 1 |
| MCM1 |  |  | MCM1 |  |  | MCM1 | 2 |
| MGA2 |  | MGA2 | MGA2 |  |  | MGA2 | 3 |
| MSN2 | MSN2 | MSN2 | MSN2 | MSN2 | MSN2 | MSN2 | 6 |
| MSN4 |  |  |  | MSN4 |  | MSN4 | 2 |
| RDS3 |  | RDS3 | RDS3 |  |  |  | 2 |
| SFP1 | SFP1 | SFP1 | SFP1 | SFP1 | SFP1 |  | 5 |
| STB5 |  | STB5 | STB5 |  | STB5 |  | 3 |
| SWI4 | SWI4 | SWI4 | SWI4 | SWI4 | SWI4 | SWI4 | 6 |
| SWI5 |  | SWI5 | SWI5 | SWI5 | SWI5 |  | 4 |
| TEC1 |  |  |  | TEC1 |  |  | 1 |
| YHP1 | YHP1 | YHP1 | YHP1 | YHP1 | YHP1 |  | 5 |
| YOX1 | YOX1 | YOX1 | YOX1 | YOX1 | YOX1 |  | 5 |
| ZAP1 | ZAP1 |  | ZAP1 |  | ZAP1 | ZAP1 | 4 |

* MSN2 and SWI4 were included in each of the six database-derived networks based on empirical evidence. CIN5, GLN3, HAP4, and HMO1 were manually included in all six GRNs, as deletion strain data was obtained from yeast in which these genes were knocked out.
* AFT2 and ASF1 only appear in db1.
* CYC8 and TEC1 only appear in db4.
* CST6 and HSF1 only appear in db6.
  + Further, SFP1, YHP1, and YOX1 are only *excluded* in db6.
* db1 and db6 (see above) had the highest theoretical minimum LSE values of the six database-derived networks.

**Table 3.** Characterization of weighted edges in db1-db6.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **db1** | **db2** | **db3** | **db4** | **db5** | **db6** |
| # Activation | 19 | 6 | 18 | 16 | 14 | 13 |
| # Repression | 7 | 11 | 10 | 12 | 10 | 11 |
| # Small Influence | 10 | 8 | 4 | 7 | 4 | 3 |
|  |  |  |  |  |  |  |
| **SUM:** | 8.1432 | -13.2390 | 1.5688 | -2.5750 | 1.2985 | -5.8876 |
| **SUM(abs):** | 38.8426 | 27.1541 | 36.1524 | 36.9479 | 34.2000 | 33.0703 |
| **MAX:** | 5.9424 | 3.0815 | 3.1810 | 2.4940 | 3.2167 | 3.7631 |
| **MIN:** | -3.3601 | -4.2947 | -3.6707 | -4.0281 | -4.0374 | -3.5141 |
| **MAX(abs):** | 5.9424 | 4.2947 | 3.6707 | 4.0281 | 4.0374 | 3.7631 |
| **SI Threshold:** | 0.2971 | 0.2147 | 0.1835 | 0.2014 | 0.2019 | 0.1882 |
| **MEAN:** | 0.2262 | -0.5296 | 0.0490 | -0.0736 | 0.0464 | -0.2181 |
| **STDEV:** | 1.6215 | 1.5085 | 1.5597 | 1.5026 | 1.6433 | 1.6812 |
| **MEDIAN:** | 0.3310 | -0.1214 | 0.2323 | 0.1139 | 0.2144 | 0.1276 |

* In **db1**, there was **substantially** **more activation than repression** of genes in the network.
  + Edge counts: 19 activation vs. 7 repression
  + Sum of edge weights = 8.1432
  + Mean edge weight = 0.2262
* In **db2**, there was **substantially** **more repression that activation** of genes in the network.
  + Edge counts: 6 activation vs. 11 repression
  + Sum of edge weights = -13.2390
  + Mean edge weight = -0.5296
* db1 and db2 exhibited a large number of small influence regulatory relationships (~30%), which we define as edges with regulatory weights < 5% of the maximum edge weight in the network. This contrasts to db3, db5, and db6, where > 15% of edges were classified as small influence.
  + In db1, the greatest activation relationship exhibited an edge weight of 5.9424, which far exceeded the weight of any other activation relationship in db2-db6 (next highest: 3.7631, db6).
    - The regulatory relationship in question is MSN2 🡪 AFT2.
    - Note: AFT2 only appears in db1, where MSN2 is its only input.
    - 
  + In db2, the greatest repression relationship exhibited an edge weight of -4.2947, which was the highest magnitude repression relationship of any network.
    - The regulatory relationship in question is SWI4 🡪 YHP1.
    - Note: This relationship appears in three other networks, although with lower magnitudes (and as a weak activation relationship in the case of db1).
    - 
  + These outliers contribute to the observed increase in “small influence” edges in db1 and db2. They might also influence the large imbalance of activation and repression in these two networks.
* In **db3-db5**, there were more activation than repression regulatory relationships, although the **overall magnitudes of activation and repression in the networks are similar**.
  + Activating edges: 18, 16, 14
  + Repressing edges: 10, 12, 10
  + Sum of edge weights = 1.5688, -2.5750, 1.2985
  + Mean edge weights = 0.0490, -0.0736, 0.0464
* In **db6**, there were similar numbers of activation and repression regulatory relationships, although the **overall magnitude of repression exceeded that of activation in the network**.
  + Edge counts: 13 activation vs. 11 repression
  + Sum of edge weights = -5.8876
  + Mean edge weight = -0.2181
* For db2-db5, the edge weights with the largest magnitudes cluster together in a relatively tight range: 3.6707-4.2947.

**Table 4.** Heat map comparing the edge weights of regulatory relationships in db1-db6.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **db1** | **db2** | **db3** | **db4** | **db5** | **db6** |  | **db1** | **db2** | **db3** | **db4** | **db5** | **db6** |
| ABF1->ASF1 |  |  |  |  |  |  | MCM1->SWI4 |  |  |  |  |  |  |
| ABF1->ASH1 |  |  |  |  |  |  | MCM1->SWI5 |  |  |  |  |  |  |
| ABF1->CST6 |  |  |  |  |  |  | MCM1->YHP1 |  |  |  |  |  |  |
| ABF1->HMO1 |  |  |  |  |  |  | MCM1->YOX1 |  |  |  |  |  |  |
| ABF1->MGA2 |  |  |  |  |  |  | MSN2->AFT2 |  |  |  |  |  |  |
| ABF1->MSN4 |  |  |  |  |  |  | MSN2->ASF1 |  |  |  |  |  |  |
| ABF1->SFP1 |  |  |  |  |  |  | MSN2->ASH1 |  |  |  |  |  |  |
| ABF1->SWI4 |  |  |  |  |  |  | MSN2->CIN5 |  |  |  |  |  |  |
| ACE2->ASH1 |  |  |  |  |  |  | MSN2->CYC8 |  |  |  |  |  |  |
| ASH1->YHP1 |  |  |  |  |  |  | MSN2->HAP4 |  |  |  |  |  |  |
| CIN5->ASF1 |  |  |  |  |  |  | MSN2->MSN4 |  |  |  |  |  |  |
| CIN5->CYC8 |  |  |  |  |  |  | MSN2->SFP1 |  |  |  |  |  |  |
| CIN5->HAP4 |  |  |  |  |  |  | MSN2->SWI4 |  |  |  |  |  |  |
| CIN5->SFP1 |  |  |  |  |  |  | MSN2->TEC1 |  |  |  |  |  |  |
| CIN5->STB5 |  |  |  |  |  |  | MSN2->YHP1 |  |  |  |  |  |  |
| CIN5->TEC1 |  |  |  |  |  |  | MSN2->YOX1 |  |  |  |  |  |  |
| CIN5->YHP1 |  |  |  |  |  |  | SFP1->SWI5 |  |  |  |  |  |  |
| GCN4->GLN3 |  |  |  |  |  |  | STB5->HAP4 |  |  |  |  |  |  |
| GLN3->GCN4 |  |  |  |  |  |  | STB5->SFP1 |  |  |  |  |  |  |
| GLN3->MGA2 |  |  |  |  |  |  | SWI4->CYC8 |  |  |  |  |  |  |
| GCR2->MSN2 |  |  |  |  |  |  | SWI4->HAP4 |  |  |  |  |  |  |
| HAP4->GCN4 |  |  |  |  |  |  | SWI4->YHP1 |  |  |  |  |  |  |
| HMO1->ASF1 |  |  |  |  |  |  | SWI4->YOX1 |  |  |  |  |  |  |
| HMO1->CIN5 |  |  |  |  |  |  | SWI5->ASH1 |  |  |  |  |  |  |
| HMO1->CYC8 |  |  |  |  |  |  | SWI5->TEC1 |  |  |  |  |  |  |
| HMO1->GCN4 |  |  |  |  |  |  | TEC1->CIN5 |  |  |  |  |  |  |
| HMO1->HAP4 |  |  |  |  |  |  | TEC1->HAP4 |  |  |  |  |  |  |
| HMO1->HMO1 |  |  |  |  |  |  | TEC1->MSN2 |  |  |  |  |  |  |
| HMO1->HSF1 |  |  |  |  |  |  | TEC1->SFP1 |  |  |  |  |  |  |
| HMO1->MCM1 |  |  |  |  |  |  | TEC1->YHP1 |  |  |  |  |  |  |
| HMO1->MSN2 |  |  |  |  |  |  | YHP1->ASF1 |  |  |  |  |  |  |
| HMO1->MSN4 |  |  |  |  |  |  | YHP1->GLN3 |  |  |  |  |  |  |
| HMO1->TEC1 |  |  |  |  |  |  | YHP1->RDS3 |  |  |  |  |  |  |
| HMO1->YOX1 |  |  |  |  |  |  | ZAP1->ACE2 |  |  |  |  |  |  |
| MCM1->ACE2 |  |  |  |  |  |  |  |  |  |  |  |  |  |



**Maximum: 1**

**Minimum: -1**

* The following regulatory relationships were conserved across the database-derived networks, always appearing as activation or repression only when present:
  + Activation, CIN5🡪HAP4 (6 networks)
  + Activation, HMO1🡪CIN5 (6 networks)
  + Activation, HMO1🡪HMO1 (6 networks)
  + Activation, HMO1🡪MSN2 (6 networks)
  + Activation, HMO1🡪YOX1 (5 networks)
  + Activation, YHP1🡪GLN3 (5 networks)
  + Activation, ZAP1🡪ACE2 (4 networks)
  + Repression, SWI4🡪HAP4 (6 networks)
  + Repression, SFP1🡪SWI5 (4 networks)
  + The conserved activation relationships among CIN5, GLN3, HMO1, and YHP1 form the following regulatory chain: HMO1 🡪 CIN5 🡪 YHP1 🡪 GLN3.
* The following high magnitude edge weights appeared in the database-derived networks both as activation and repression relationships:
  + GCR2🡪MSN2 (2 Activation, 3 Repression)
    - Note: MSN2 is the only connection to GCR2 in these networks.
  + MSN2🡪CIN5 (2 Activation, 4 Repression)
  + MSN2🡪HAP4 (3 Activation, 3 Repression)
  + MSN2🡪SFP1 (3 Activation, 2 Repression)
  + MSN2🡪SWI4 (3 Activation, 3 Repression)
  + MSN2🡪YHP1 (3 Activation, 2 Repression)
  + MSN2🡪YOX1 (3 Activation, 2 Repression)
  + SWI4🡪YHP1 (2 Activation, 3 Repression)
  + SWI4🡪YOX1 (1 Activation, 2 Repression)
  + MCM1🡪SWI4 (1 Activation, 1 Repression)
  + All but one of these regulatory relationships involve either MSN2 or YHP1, which both exhibit high betweenness centralities.

[MSN2: high betweenness centrality, gatekeeper of downstream dynamics, dynamic modeling.

vs. HMO1… originator of dynamics, well-conserved?]

db1-db6.pdf