

ANALYSIS OF RNA-SEQ DATA FOR RAP1:

RAP1:

- ▶ General Regulatory Factor¹
 - ▶ Abundant + essential.
 - ▶ Binding sites found in many promoters.
 - ▶ Enhancers of regulation.
- ▶ According to SGD, targets 1655 genes
- ▶ Chromatin silencing²
- ▶ Regulation of ribosome components³

¹Fourel et al., 2002

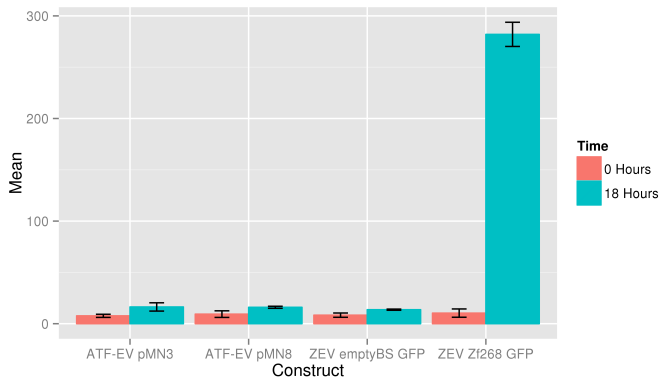
²Konig et al., 1996

³Lieb et al., 2001

FIRST DISCLAIMER:

RNA-seq data for only the ATFs for RAP1 was generated during the first 3 weeks of classes.

SECOND DISCLAIMER:



Number of GFP positive cells by FACS

CuffDiff run to compare:

- ▶ 0" to 15"
- ▶ 0" to 90"
- ▶ 0" min to 90" uninduced
- ▶ 15" to 90"
- ▶ 15" to 90" uninduced
- ▶ 90" to 90" uninduced

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The only significant differences found were between **0 and 90" induced**.

	Gene	Log2 Fold Change	p-value
1	TIS11	2.69	5×10^{-5}
2	INO1	2.50	5×10^{-5}
3	SSA2	-1.90	5×10^{-5}
4	YEL073C	1.74	5×10^{-5}
5	GPG1	1.52	5×10^{-5}
6	ICY1	1.39	5×10^{-5}
7	MRS3	1.35	5×10^{-5}
8	YOR387C	1.27	5×10^{-5}
9	PDR12	1.26	5×10^{-5}
10	GSC2	1.25	5×10^{-5}
11	YMR090W	1.24	5×10^{-5}

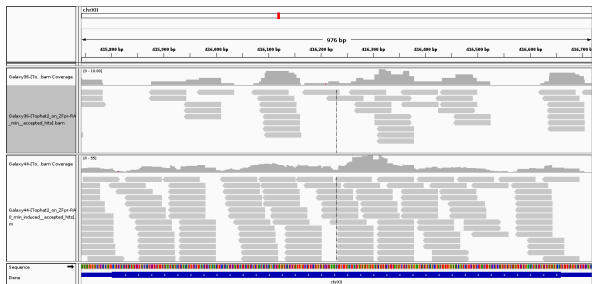
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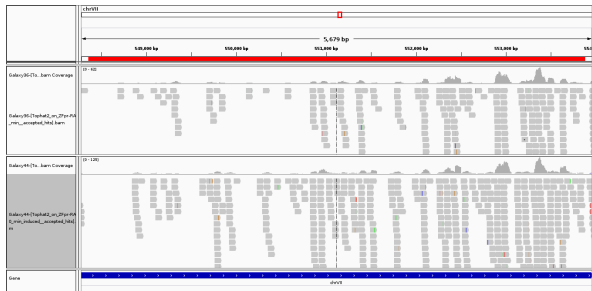
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TIS11:



GSC2



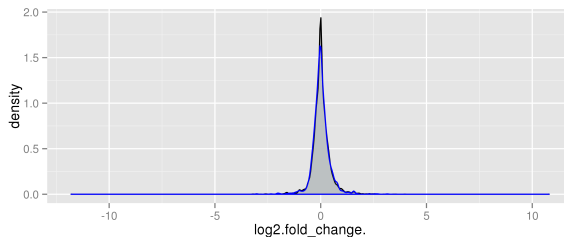


Figure : 0 - 90 minutes induced. Distribution of fold change for all genes, **SGD targets**.

Wilcoxon significance:

- **All vs. SGD targets:** $p\text{-value} = 0.2101$.

There is no difference in fold change between Rap1 targets and the full set of genes.

Yarragudi et al, 2007 - provides a list of probable and putative Rap1 targets by experimentation with Rap1 ts mutants.

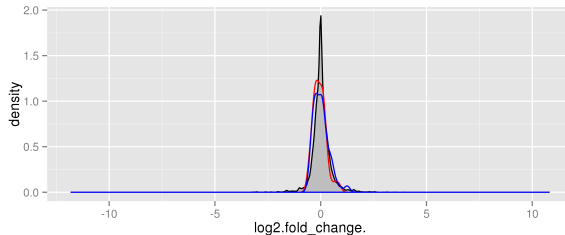


Figure : 0 - 90 minutes induced. Distribution of fold change for all genes, putative direct Rap1 targets, and probable direct Rap1 targets from Yarragudi et al, 2007.

Wilcoxon significance:

- ▶ **All vs. Putative:** p-value = 0.5316.
- ▶ **All vs. Probable:** p-value = 0.08193.

Where do the top hits come from?

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	Gene	Function
1	TIS11	Increases on DNA replication stress
2	INO1	Inositol phosphate synthesis
3	SSA2	Stress response / Protein folding
4	YEL073C	Unknown function, regulated by Inositol
5	GPG1	Signal Transduction
6	ICY1	Unknown function, paralog to ICY2
7	MRS3	Iron transporter (mitochondrion)
8	YOR387C	Unknown function, has paralog VEL1
9	PDR12	Membrane transporter
10	GSC2	Spore wall formation
11	YMR090W	Unknown function

ENRICHED GO TERMS, 0 TO 90" INDUCED:

GO term	p-value
RNA modification guide activity	8.194×10^{-09}
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base pairing with rRNA	1.228×10^{-08}
rRNA modification	1.719×10^{-07}
small nucleolar ribonucleoprotein complex	1.609×10^{-06}

ENRICHED GO TERMS, 0 TO 15" INDUCED:

GO term	p-value
cytosolic large ribosomal subunit	3.1129×10^{-37}
cytosolic small ribosomal subunit	8.9788×10^{-24}
cytosolic ribosome	9.6384×10^{-57}
rRNA export from nucleus	3.4673×10^{-07}
rRNA transport	3.4673×10^{-07}
glycolysis	2.1990×10^{-09}
cytoplasmic translation	5.8050×10^{-44}
cytosolic part	1.3412×10^{-43}
glucose catabolic process	1.6974×10^{-10}
structural constituent of ribosome	1.5817×10^{-31}
large ribosomal subunit	1.8137×10^{-19}
hexose catabolic process	5.4872×10^{-09}
ribosomal subunit	6.0681×10^{-31}
monosaccharide catabolic process	4.4285×10^{-09}
small ribosomal subunit	1.9764×10^{-12}
ribosome	1.9820×10^{-27}
pyridine nucleotide metabolic process	4.3267×10^{-05}
glucose metabolic process	2.7083×10^{-06}
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Lieb et al., 2001 - almost all ($124/137$) ribosomal protein genes (RPG) have binding sites for Rap1.

Using ~ 300 genes classified under the ribosome cell component in gene ontology:

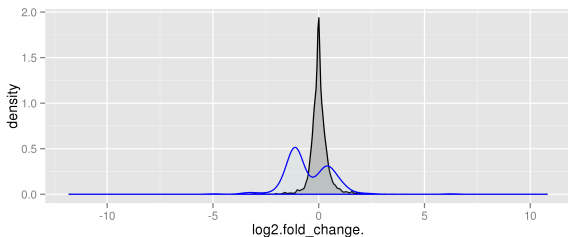


Figure : 0 - 15 minutes induced. Distribution of fold change for all genes, all ribosome genes

RPGs seem to be downregulated by overexpression of Rap1.
Wilcoxon significance:

- **All vs. Ribosomal** p-value = 2.2×10^{-16}

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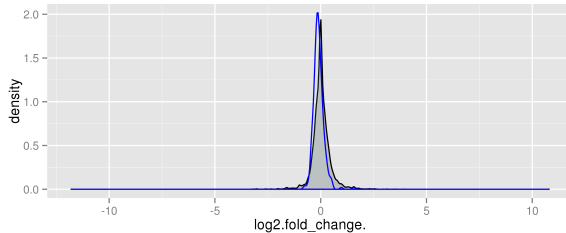


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RPGs seem to be downregulated by overexpression of Rap1.
Wilcoxon significance:

- ▶ **All vs. Ribosomal** p-value = 7.899×10^{-16}

Using a different transcription factor (MIG1):

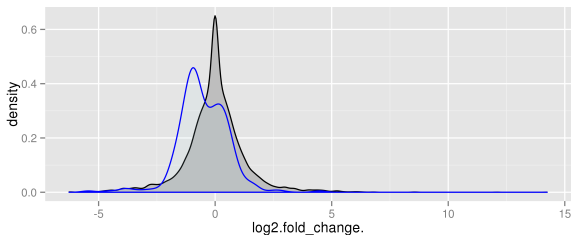
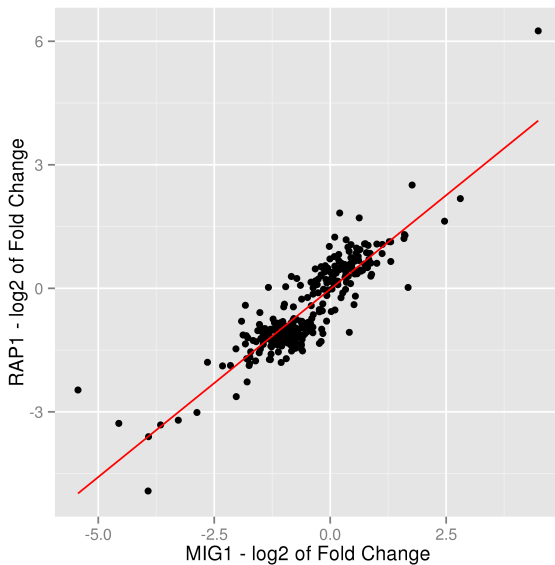


Figure : 0 - 15 minutes induced for MIG1. Distribution of fold change for all genes, **all ribosome genes**

Wilcoxon significance:

- ▶ **MIG1 all vs. MIG1 ribosomal** p-value = 2.2×10^{-16}



MIG1 v. RAP1 0-15 Ribosome

Adjusted $R^2 = 0.7816$.

Genes w/ low p-values from 0 to 15 minutes (*immediate response*) are highly enriched for stress response: *DDR2*, *SSA2*, *PHM7*, *TMA10*, *HSP26*...

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Why might the yeast stress response be activated?

- (1) **Freeman et al., 1995** - overexpression of Rap1 in yeast is toxic.
- (2) The ATF-EV construct may be toxic.
- (3) **Banerjee et al., 2004** show that progesterone activates the stress response (however, β -estradiol is native, progesterone is not).

$68/152$ genes under “response to stress” are also Rap1 Targets. (p-val = 0.00136 by χ^2)

Is the stress response activated?

Look at 0 to 15 minutes induction. The ~ 150 genes annotated under GO term *response to stress* are, on average, upregulated between 0 and 15 min.

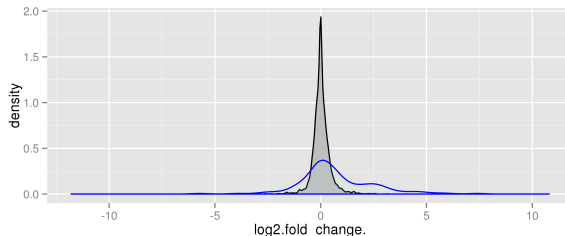


Figure : 0 - 15 minutes induced. Distribution of fold change for all genes, **stress response genes**.

Wilcoxon significance:

- **All vs. Stress Response:** $p\text{-value} = 3.031 \times 10^{-08}$

Is the stress response a β -estradiol or otherwise technical response?

0 to 15 minutes induction for another transcription factor (MIG1).

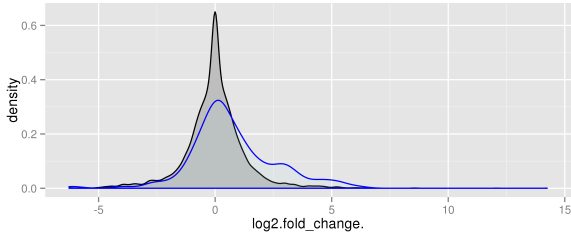
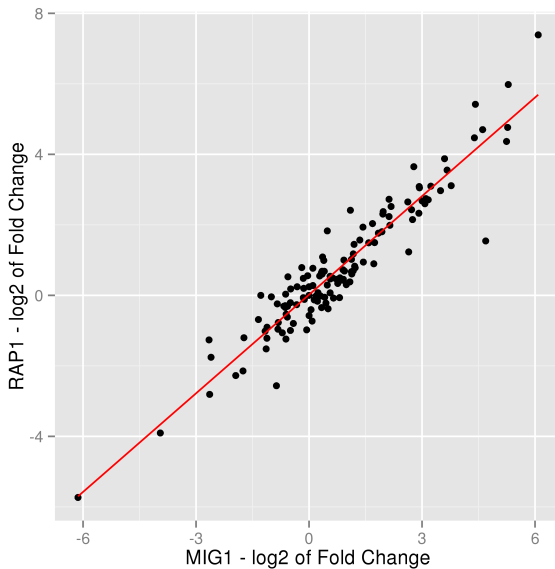


Figure : 0 - 15 minutes induced for MIG1. Distribution of fold change for all genes, **stress response genes**.

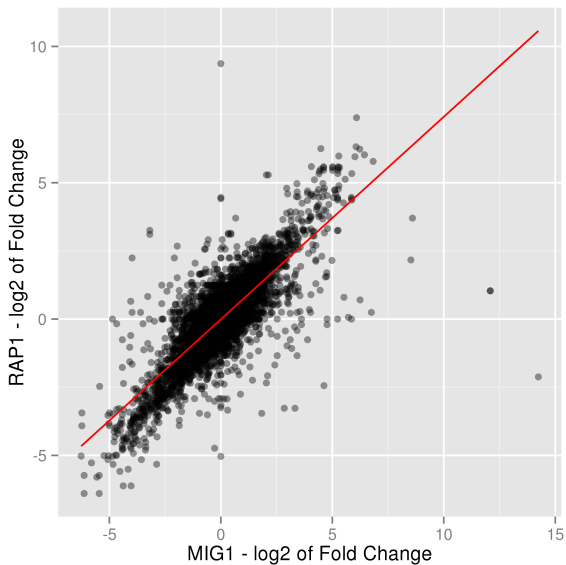
Wilcoxon significance:

- **MIG1 All vs. MIG1 Stress Response:** p-value
 $= 9.934 \times 10^{-08}$



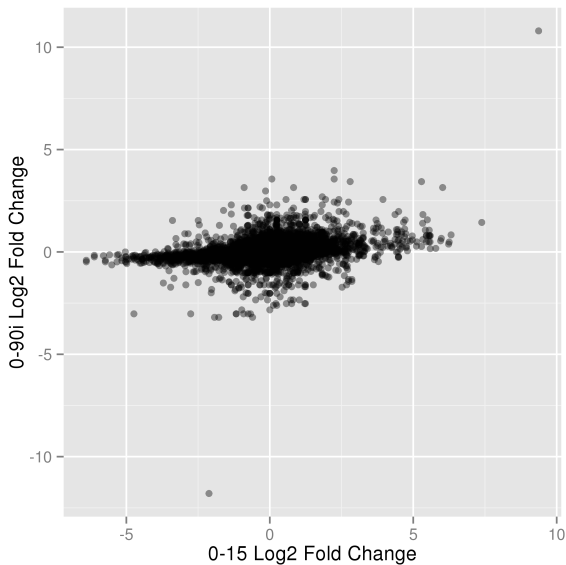
MIG1 v. RAP1 0-15 Stress Response

Adjusted $R^2 = 0.8914$.



MIG1 v. RAP1 0-15 All

Adjusted $R^2 = 0.6428$.



RAP1 0 to 15 v. 0 to 90 All

Adjusted $R^2 = 0.09552$.

CONCLUSIONS

- ▶ Clear fast stress response after adding β -estradiol.
- ▶ Ribosomal down-regulation in contradicts what we know about Rap1 targeting.
- ▶ Stress may also lead to decreased ribosome expression.
- ▶ Coordinated response after 15 minutes in both MIG1 and RAP1.
- ▶ ATF may not have worked.
- ▶ Because Rap1 is a GRF, it may need TF partners to activate/repress.