Investigating the Quantitative Relationship Between Transcription Factor and Target in Yeast

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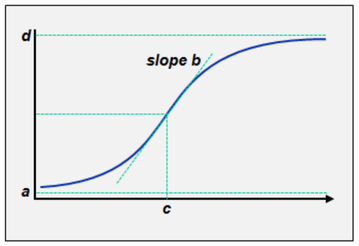
Hill Function as a Model for Dose-Response Curves

(1) $$F(x) = d + \frac{(a-d)}{1+(\frac{x}{c})^b}$$

Gene of Interest FPKM

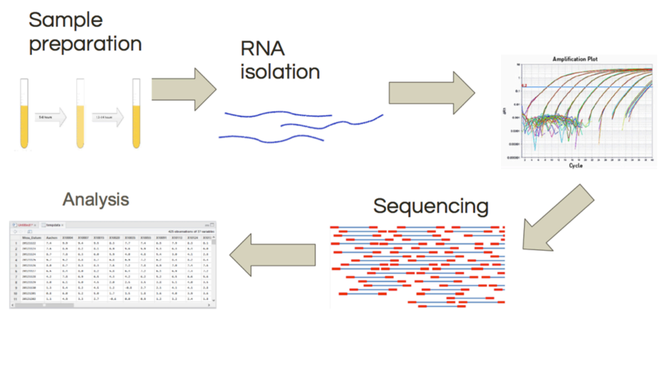


(2) $$n_H = \frac{\log_{10}(81)}{\log_{10}(EC_{90}/EC_{10})}$$

a = Min
b = Slope
c = EC50
d = Max

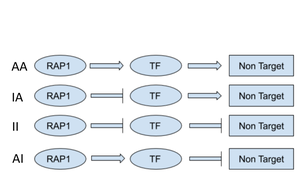
The Log-Logistic equation (1) used to model the parameters in the hill curve is above. Models generated using the 'drc' R package. Equation 2 is to generate Hill Coefficients (n_H).

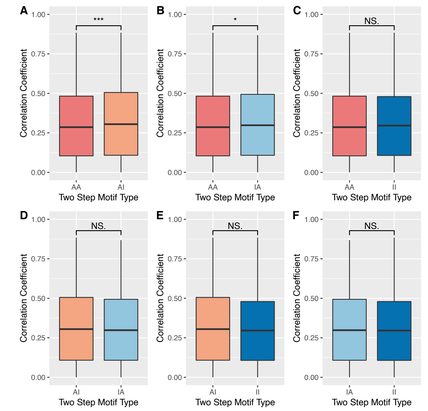
Experimental Design



Yeast were grown in liquid culture in replicate with varying levels of dox to titrate RAP1 expression, RNA was isolated and used to build RNA sequencing libraries.

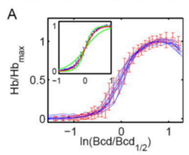
Two Step Motifs Stemming from RAP1



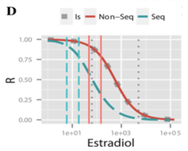


Network motifs are repeated patterns of interactions between nodes in a network. The network motifs are shown above and the significance of the network motifs on the correlation of the non target expression and RAP1 expression is shown to the right. Correlation coefficient corresponds to Spearmen's correlation coefficient.

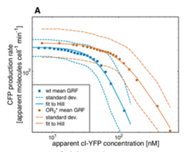
Dose-Response Relationships Modeled by Hill Functions in Literature



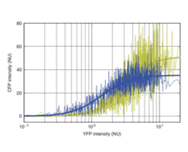
Gregor et al. *Cell*, 2007



Altszyler et al. *PLOS*, 2017



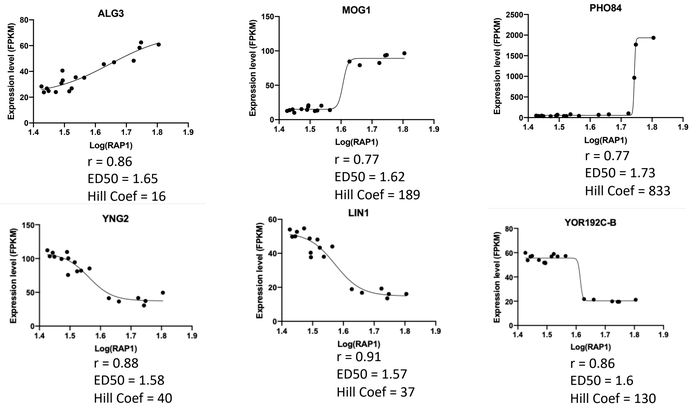
Rosenfeld et al. *Science*, 2005



Kim et al. *Nat Struct Mol Biol*, 2008

Transcriptional regulation is a critical step in most biological processes. Most models of transcription factor-target relationships do not include transcription factor abundance. Above are the four examples from literature beginning to quantify this relationship. We extended the above work by quantifying genome-wide gene expression and using RAP1 expression levels along with its targets expression levels to begin to quantify the complicated relationship between transcription factor and target.

RAP1-Target Relationships Modeled by Hill Function Parameters



The curves showing RAP1 acting as an activator (top row) or inhibitor (bottom row).

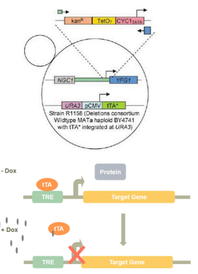
Indegree Effect on Correlation with RAP1 Expression

The indegree of a gene corresponds with the number of transcription factors that regulate the gene. All genes shown are directly regulated by at least RAP1. Correlation coefficient corresponds to Spearmen's correlation coefficient.

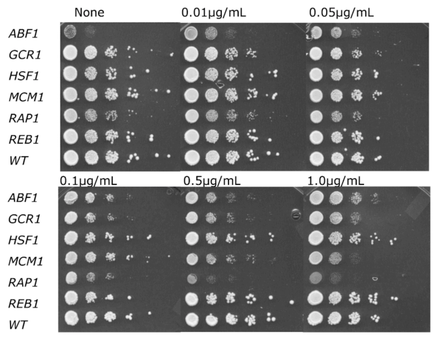
Conclusions

- Indegree does not influence correlation with RAP1 expression suggesting the identity of the regulators is more important then quantity.
- The hill function parameters identified by this model are variable suggesting a more complex relationship between transcription factor and target than previously thought.
- The identity of the edge that connects RAP1 to a transcription factor, and the transcription factor to its downstream targets, matters in the correlation of that gene's expression with RAP1 expression.
- Direct targets of RAP1 expression levels are better correlated with RAP1 expression than non-direct targets

Titrating Transcription Factors Affects Growth Phenotype

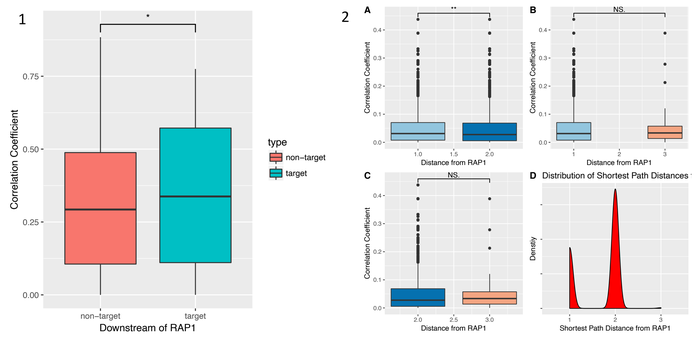


Mnaimneh et al. *Cell*. 2004



Strains obtained from Yeast Tet-promoter Hughes collection and contain a Tetracycline Response Element (TRE) inserted in the promoter region of the gene of interest. A frogging assay (right) was used to observe differences between growth phenotypes of different yeast strains. The yeast were grown on a 96 well plate containing varying levels of Doxycycline.

Distance from RAP1 Effect on Correlation with RAP1 Expression



(1) Direct targets of RAP1 are better correlated with RAP1 expression than non-direct targets. (2) Plots showing significance between 1 and 2 hops from RAP1 (A), 1 and 3 hops (B) and 2 and 3 hops (C). (D) Distribution of shortest path distances from RAP1. Correlation coefficient corresponds to Spearmen's correlation coefficient.

Future Directions

- Extend model to transcription factors: ABF1, MCM1, HSF1, GCR1, REB1
- Extend model to more environments
- Perform ChIP-seq for RAP1 binding sites

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