Model-Based Quantification of Induced Gene Expression Time Courses

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INTRODUCTION

We model gene expression time courses following induction of a glucocorticoid receptor-bound transcription factor into the nucleus due to exposure to dexamethasone in Arabidopsis thaliana.

In contrast to measurement of induced expression at a single time, resulting in a single fold-change value typically long after the induction event, we model the dynamics of TF import into the nucleus followed by the transcriptional dynamics of its direct and indirect targets.

The model quantifies an observed time course with two rate coefficients:

1. $\hat{\eta}_p$ = the normalized initial rate of rise (+) or fall (-) of target mRNA levels
2. γ_p = the mRNA loss rate

These two processes compete to determine the final transcript fold change: a highly-induced transcript may saturate at a low fold change if it also decays quickly, while weakly-induced transcripts may rise to a high level if they have very low loss.

In addition, some targets exhibit "turn-off", or autoregulation. These time courses require a turn-off time, t_{off}, after which the mRNA level decays exponentially at γ_p.

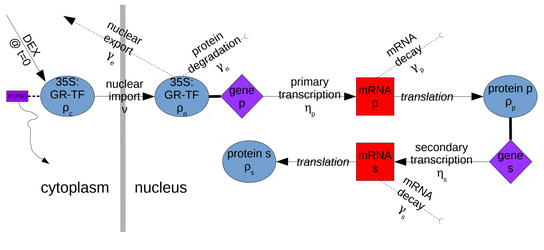
We have conducted experiments with GR-TFs involved in leaf regulation: *SHOOT MERISTEMLESS/STM* (AT1G65620); *ASYMMETRIC LEAVES 2/AS2/LBD6* (AT1G65620); *KANADI 1/KAN1* (AT5G16560); *REVOLUTA/REV* (AT5G60690); and *TINY* (AT5G25810).

Our experiments measure transcript time courses for up to two hours after DEX exposure with both RNA-seq and microarray assays. Since secondary targets of the induced transcription factor are expected to rise after some delay, during which the primary transcript's protein level is rising, we explore the use of our model to distinguish between primary and secondary targets.

OBJECTIVES

1. Model the import of the GR-TF into the nucleus after DEX exposure
2. Model the modified transcription of GR-TF primary targets (subscript p)
3. Model the modified transcription of GR-TF secondary targets (subscript s), which are primary targets of GR-TF primary targets
4. Compare the model-fitted rate coefficients with standard fold-change analysis

MODEL AND EXPERIMENTAL METHOD



Nuclear import model

Transport of a glucocorticoid receptor (GR) into the nucleus after DEX application has been measured in animal tissue by several groups.

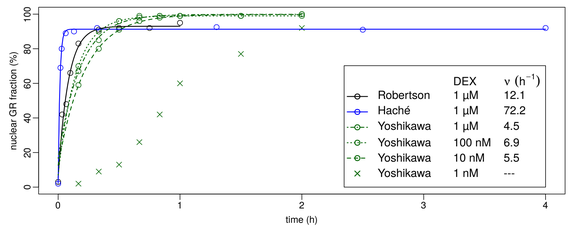


Figure 1: The measured nuclear GR concentration follows a saturated exponential time course, except at very low DEX concentration. Data sources in References.

The measured time courses are well described by a pair of linear ODEs:

$$\dot{\rho}_c = -\nu\rho_c + \gamma_e\rho_n$$
$$\dot{\rho}_n = \nu\rho_c - \gamma_e\rho_n - \gamma_n\rho_n$$

ρ_c = cytoplasmic GR protein concentration
ρ_n = nuclear GR protein concentration
ν = nuclear import rate
γ_e = nuclear export rate
γ_n = rate of loss of GR-TF from other causes

We neglect nuclear export and loss setting $\gamma_e = 0$ and $\gamma_n = 0$ since those rates are slow compared to our two-hour measurement span. We assume that import of chimeric GR-TF is similar to the published GR import data.

Transcription model

We model altered transcription of target mRNA due to DEX-induced import of the GR-TF with a set of coupled ODEs:

$$\dot{\rho}_p = \eta_p\rho_n - \gamma_p\rho_p$$
$$\dot{\rho}_s = \eta_s\rho_p - \gamma_s\rho_s$$

ρ_p = expressed mRNA/protein of a primary target of the GR-TF
ρ_s = expressed mRNA/protein of a secondary target of the GR-TF
γ_p = loss rate of primary mRNA/protein
γ_s = loss rate of secondary mRNA/protein
η_p = transcription rate between the GR-TF and primary target
η_s = transcription rate between the primary and secondary targets

We assume that mRNA and protein concentrations are in fixed proportion, so that ρ_p and ρ_s represent measured mRNA levels as well as protein concentration. (This avoids two more equations and four more fit parameters accounting for translation.) γ_p and γ_s represent loss of mRNA.

These equations can be solved analytically; we set $\nu = 10\text{h}^{-1}$ based on the animal tissue studies (*we need to measure it!*).

Primary target expression time course metrics

The model provides two independent time course metrics:

- $\hat{\eta}_p \equiv \eta_p\rho_n(0)/\rho_p(0)$ characterizes the *initial change* of mRNA level.
- γ_p characterizes the *loss* of mRNA.

In addition, we derive an asymptotic $t\to\infty$ fold change:

$$\text{logFC}_\infty \equiv \lim_{t\to\infty} \log_2 \frac{\rho_p(t)}{\rho_p(0)} = \log_2\left(1 + \frac{\hat{\eta}_p}{\gamma_p}\frac{\rho_c(0)}{\rho_n(0)}\right)$$

The ratio $\hat{\eta}_p/\gamma_p$ therefore determines the asymptotic fold change of a transcript: **mRNA transcription and loss play equal roles in the final transcript level**.

Time-course DEX-induced GR-TF expression measurements in *Arabidopsis thaliana*

The WT and five GR-TF Col-0 plant lines were grown to seedlings, and tissue was exposed to 50 μM of DEX and flash frozen 0, 0.5, 1 and 2 hours later. Almost all GR-TF samples had three biological replicates per time (WT had six). RNA was extracted and assayed with the Affymetrix ATH1 microarray and Illumina HiSeq RNA-seq. The microarray data were analyzed using the R packages `affy` and `limma`; the RNA-seq data were analyzed using TopHat2, Cufflinks and Cuffdiff. Sequences were mapped to the TAIR10 reference genome.

RESULTS

Time courses exhibit qualitative variation

<u>When</u> you measure DE in an induced expression experiment is important!

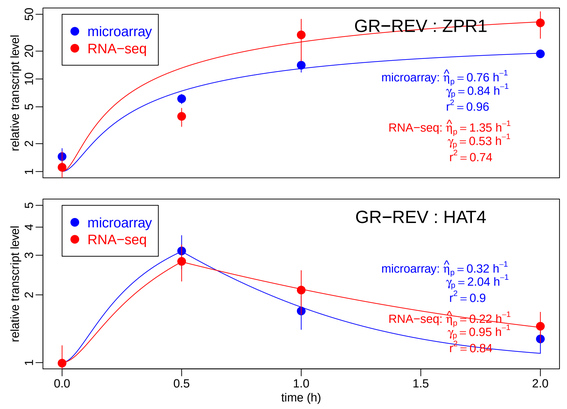


Figure 2: Expression time courses vary greatly, from strong GR-REV monotonic risers like *ZPR1* to HD-ZIPII self-regulators like *HAT4*. Lines represent primary target model fits with transcription turned off at t=30 mins for *HAT4*. (*HAT4* turn-off occurs some time between t=15 and 45 mins.)

Model parameters $\hat{\eta}_p$ and γ_p distinguish time course shapes

The induced expression change can be large due to high transcription rate and/or low transcript loss. Asymptotic fold change differs greatly from that measured at individual times after DEX application.

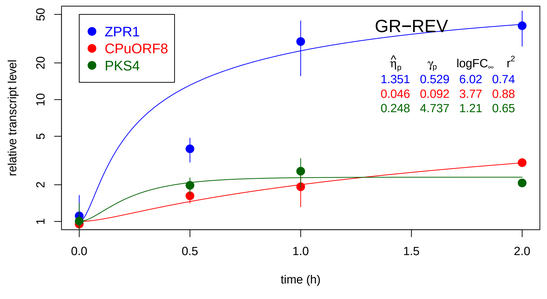


Figure 3: Distinct time course shapes result from different transcription strength vs. loss rate for these GR-REV targets: high $\hat{\eta}_p$, low γ_p for *ZPR1*, the most strongly regulated GR-REV target; low $\hat{\eta}_p$, low γ_p for *CPuORF8*, which reaches a significant fold-change due to low loss; and high $\hat{\eta}_p$, high γ_p for *PKS4*, which saturates quickly due to high loss. (RNA-seq data.)

Down-regulated transcripts are also well fit

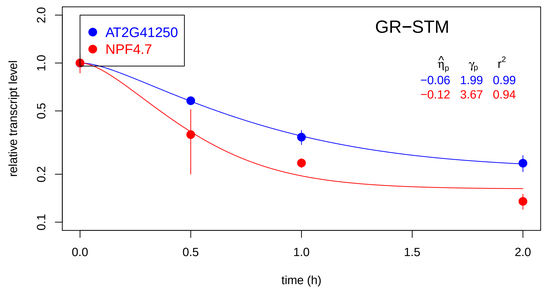


Figure 4: *GR-STM* has many down-regulated targets; these two are particularly well fit by the model. (RNA-seq data.)

Example: A down-regulated target of *HAT22*?

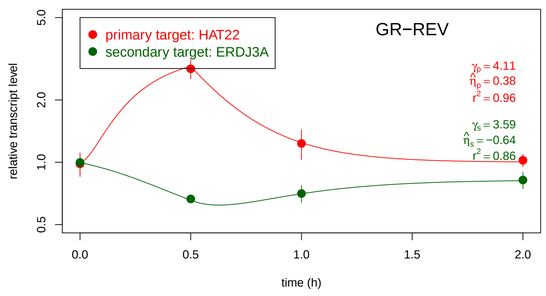


Figure 5: *HAT22* is a self-regulating target of *REV* which is also a TF. *ERDJ3A* may be a *down*-regulated target of *HAT22* which returns to its WT level after *HAT22* turns off. (Microarray data.)

CONCLUSIONS

1. DEX-induced expression of GR-TF targets exhibits a variety of time courses, requiring better quantification than single-time fold change.
2. Time courses can be fit by a basic ODE model with three shape parameters describing transcription strength, transcript loss and, when needed, turn-off.
3. The model simulates primary and secondary transcriptional responses, supporting target discovery and inference of gene regulatory networks.

Further Research

1. Improve model to make robust fits for "black box" analysis, perhaps in a Web application
2. Study tighter time course of selected GR-TFs and targets using both RNA-seq and qPCR
3. Measure GR-TF nuclear import using flourescent probes or other technique
4. Measure mRNA induction in real time with CRISPRi activation?

References

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