Gene-network inference by message passing

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Abstract. The inference of gene-regulatory processes from gene-expression data belongs to the major challenges of computational systems biology. Here we address the problem from a statistical-physics perspective and develop a message-passing algorithm which is able to infer sparse, directed and combinatorial regulatory mechanisms. Using the replica technique, the algorithmic performance can be characterized analytically for artificially generated data. The algorithm is applied to genome-wide expression data of baker’s yeast under various environmental conditions. We find clear cases of combinatorial control, and enrichment in common functional annotations of regulated genes and their regulators.

1. Introduction

Transcriptional gene regulation is at the basis of cell development and differentiation [1]. It constitutes the important feedback mechanism from the level of proteins to the transcription of genes to mRNA, and it allows to reach differentiated gene expression patterns starting from identical genetic information. The simplest mechanisms in this context are transcriptional repression and activation of a gene by a single transcription factor (TF) which are schematically depicted in figure 1:

- In the case of a repressor, the RNA polymerase (i.e. the molecular machine transcribing genes to mRNA, abbreviated as POL in the figure) and a transcription factor (i.e. a regulatory protein) have overlapping binding sites on the DNA. Due to steric exclusion effects the presence of the TF hinders the polymerase to bind to DNA and thus to transcribe the gene. Elevated levels of TF concentration thus lead to a repression of the transcription rate of the considered gene.

- In the case of an activator, polymerase and TF bind cooperatively to the DNA. Their binding sites are close, and both proteins show some attractive short-range interaction of a few $kT$. A high TF concentration thus increases the binding probability of polymerase, and transcription is enhanced.

Also the expression of transcription factors is regulated by other transcription factors. The set of all genetic interactions is called the gene-regulatory network (GRN). It is considered to be a prototypical example of a complex system. GRN are characterized in particular by the following properties:

- They are sparse: Each gene is controlled only by a limited number of other genes, which is very small compared to the total number of genes present in an organism.
• Gene networks are directed: Regulatory control is obviously directed from the regulators to the regulated genes.

• Genes are regulated via combinatorial control mechanisms: The expression level of a gene frequently depends on the joint activity of various regulatory proteins. The best-known example is the Lac operon which is activated only if glucose is absent AND lactose is present.

Understanding GRN is a major task in modern biology, but its experimental determination is extremely complicated. Genome-scale networks are only known for *E. coli* [2] and baker’s yeast [3, 4], whereas for higher organisms only few functional modules are reconstructed, see *e.g.* [5, 6]. It is therefore tempting to ask in how far gene-regulatory networks can be reconstructed starting from easily accessible data – in particular from genome wide expression data measuring simultaneously the expression level of order $10^4$ RNAs (micro-arrays). However, also this task is highly non-trivial and limited by the following restrictions on the quality and quantity of available data:

• The number $M$ of available expression patterns is in general considerably smaller than the number $N$ of measured genes.

• The available information is incomplete. The expression of some relevant genes may not be recorded, and external conditions corresponding, *e.g.*, to nutrient or mineral conditions are not given. Most importantly, micro-arrays measure the abundance of mRNA, whereas gene regulation works via the binding of the corresponding proteins (TFs) to the regulatory regions on the DNA. Active protein and RNA concentrations are, however, not in a simple one-to-one correspondence.

• Data are noisy. This concerns biological noise due to the stochastic nature of underlying molecular processes, as well as the considerable experimental noise existing in current high-throughput techniques.

• Micro-arrays do not measure the expression profiles of single cells, but of bunches of similar cells. This averaging procedure may hide the precise character of the regulatory processes taking place in the single cells.

• Non-transcriptional control mechanisms (chromatin remodeling, small RNAs etc.) cannot be taken into account in expression based algorithms.

The listed points obviously lead to a limited predictability of even the most sophisticated algorithms. State-of-the-art algorithms include relevance networks measuring pairwise correlations [7, 8], ARACNe which removes false positives in relevance networks [9, 10], Bayesian networks [11, 12, 13, 14], probabilistic Boolean networks [15, 16], and module networks [17]. It is therefore very important to test various algorithms on the basis of well described data sets.
containing some or all of the before-mentioned problems: Only a critical discussion on artificial
data sets allows for a sensible interpretation of the algorithmic outcomes when run on biological
data.

In the following, we first introduce a minimal functional model which shall be used to fit
the data. This model is incorporated in a scoring function for networks, but the construction
of high-scoring solutions is a computationally hard task. In section 3 we propose therefore a
message-passing approach to heuristically solve the problem. In section 4, the algorithm is
analyzed on artificial data, and finally it is applied to biological data of yeast under various
environmental conditions in section 5. Conclusions are drawn in the last section.

2. The model
Before setting up a functional model we have to spend a few lines discussing how data look
like. Raw micro-array data require normalization procedures, a standard way is filtering out
genes having very low variability, followed by log-ratio-normalizing the remaining data. This
means that data are divided by gene specific numbers defining a reference level of expression
for this gene, and then the logarithm is taken. This guarantees that data of expression level
lower than the reference become negative, over-expression is shown by positive values. Note
also that the logarithmic transformation regularizes the data distribution: The resulting single-
gene distributions look regular since the same fold-change in over- and under-expression lead
to symmetric log-normalized numbers. Let us therefore assume that we have such log-ratio-values

\[ x_\mu^i, \quad i = 0, \ldots, N, \quad \mu = 1, \ldots, M, \quad (1) \]

for \( N + 1 \) genes measured in \( M \) distinct micro-arrays.

The task is now to go from these data back to the interactions behind it. To use a statistical-
physics analogy, starting from snapshots of the microscopic state of an Ising model we try to
infer its Hamiltonian. Note that due to the directed structure of gene networks this task can
be formally factorized over regulated genes: We can ask first, which genes have a regulatory
influence on gene 0, and how they interact combinatorially. Then we ask the same question
for the regulators of gene 1, gene 2 etc., until we reach gene N. In the following discussion we
therefore concentrate without loss of generality on one single regulated gene \(( i = 0)\), and
\( N \) potential input genes \((1 \leq i \leq N)\).

We further simplify the possible influence genes can have on the target gene 0, we aim at a
ternary classification of the influence of a gene \( i \) on 0:

\[ J_{i \rightarrow 0} = \begin{cases} -1 & \text{if gene } i \text{ represses the expression of gene } 0, \\ 0 & \text{if gene } i \text{ does not regulate gene } 0, \\ 1 & \text{if gene } i \text{ activates the expression of gene } 0. \end{cases} \quad (2) \]

As a minimal functional model, we assume that a gene is over-expressed if the overall influence
of its neighbors is beyond some threshold \( \tau \), and it is repressed if the overall influence is smaller
then \( \tau \). We therefore expect

\[ x_0^\mu > 0 \quad \leftrightarrow \quad \sum_{i=1}^{N} J_{i \rightarrow 0} x_i^\mu > \tau \quad (3) \]

to hold for as many expression patterns \( \mu = 1, \ldots, M \) as possible. In this sense, each pattern
gives a *constraint* on the coupling vector \( \vec{J} = (J_{1 \rightarrow 0}, \ldots, J_{N \rightarrow 0}) \), and the problem of finding a
good candidate vector \( \vec{J} \) can be understood as an instance of a *constraint satisfaction problem.*
Also the threshold can be inferred similarly to \( \vec{J} \), but to avoid heavy notation we set it to zero
in the following.
A cost function for this problem counts the number of errors made in (3),
\[ \mathcal{H}(\vec{J}) = \sum_{\mu=1}^{M} \Theta \left( -x_0^\mu \sum_{i=1}^{N} J_{i \rightarrow 0} x_i^\mu \right), \]  
with \( \Theta \) being the step function. Obviously, this ternary classification is over-simplified in the sense that no weak or strong repressors and activators are considered, and more complex functions like the XOR of the inputs (or an continuously-valued generalization of it) cannot be represented in this way. However, due to the before-mentioned problems with data quality and quantity we have to restrict ourselves to a not too complex class of models in order to avoid over-fitting.

As already mentioned, GRN are sparse, i.e., only a few of the \( i \in \{1, \ldots, N\} \) will have a non-vanishing coupling to gene 0. We therefore have to control also the effective coupling number
\[ N_{\text{eff}}(\vec{J}) = \sum_{i=1}^{N} |J_{i \rightarrow 0}|, \]  
which counts only the number of non-zero entries in \( \vec{J} \). An \textit{a priori} bias towards diluted graphs is also reasonable from the machine-learning standpoint. The restriction of the entropy of the search space lowers the probability of over-fitting.

3. Inference by belief propagation

The inference task is now to characterize the properties of vectors \( \vec{J} \) which are both low-cost and diluted. To achieve this we introduce a weight
\[ W(\vec{J}) = \exp \left\{ -\beta \mathcal{H}(\vec{J}) - h N_{\text{eff}}(\vec{J}) \right\}, \]  
which still depends on two external parameters \( \beta \) and \( h \) which act as a formal inverse temperature and a diluting field. The size of these parameters determines the relative importance of low-cost vectors compared to sparse ones.

In order to get information about the statistical properties of the single-gene couplings \( J_{i \rightarrow 0} \) we have to calculate marginals
\[ P_i(J_{i \rightarrow 0}) \propto \sum_{\{J_{j \rightarrow 0} \in \{0, \pm 1\}; j \neq i\}} W(\vec{J}). \]  
The probability \( P_i(J_{i \rightarrow 0} \neq 0) = 1 - P_i(0) \) of having a non-zero coupling can be used to rank genes according to their relevance for gene 0. However, the direct calculation requires a sum over \( 3^{N-1} \) configurations and is therefore infeasible even for relatively small systems. The construction of high-weight vectors itself is already an NP-hard task, so we need to apply heuristic methods to approximate \( P_i \).

The main idea is to use belief propagation (BP). Variables and constraints exchange messages,
\[ P_{i \rightarrow \mu}(J_{i \rightarrow 0}) \propto e^{-h|J_{i \rightarrow 0}|} \prod_{\nu \neq \mu} \rho_{\nu \rightarrow i}(J_{i \rightarrow 0}), \]
\[ \rho_{\mu \rightarrow i}(J_{i \rightarrow 0}) \propto \sum_{\{J_{j \rightarrow 0}; j \neq i\}} \exp \left\{ -\beta \Theta \left[ -x_0^\mu \sum_{k} J_{k \rightarrow 0} x_k^\mu \right] \right\} \prod_{j \neq i} P_{j \rightarrow \mu}(J_{j \rightarrow 0}), \]  
which have to be determined self-consistently. They can be used to calculate the BP approximation for the marginal distributions,
\[ P_i(J_{i \rightarrow 0}) \propto e^{-h|J_{i \rightarrow 0}|} \prod_{\mu} \rho_{\mu \rightarrow i}(J_{i \rightarrow 0}). \]
Looking a bit closer to the second of equations (8), we see that it still contains the exponential summation over coupling configurations. Due to the factorization of messages used in BP it results in an average over independent random variables. The quantity to be averaged depends only on the sum over these variables, so we may use a Gaussian approximation

$$\rho_{\mu \rightarrow i}(J_{i \rightarrow 0}) \propto \int_{-\infty}^{\infty} \frac{dh}{\sqrt{2\pi} \Delta_{\mu \rightarrow i}} \exp \left\{ -\frac{(h - h_{\mu \rightarrow i})^2}{2\Delta_{\mu \rightarrow i}^2} - \beta \Theta \left[ -x_0^\mu \cdot (J_{i \rightarrow 0} x_i^\mu + h) \right] \right\}$$

(10)

with

$$h_{\mu \rightarrow i} = \sum_{j \neq i} x_j^\mu \langle J_{j \rightarrow 0} \rangle_{j \rightarrow \mu} ,$$

$$\Delta_{\mu \rightarrow i}^2 = \sum_{j \neq i} \left( \langle J_j^2 \rangle_{j \rightarrow \mu} - \langle J_{j \rightarrow 0} \rangle_{j \rightarrow \mu}^2 \right) ,$$

(11)

which brings the computational cost for a single message update down to linear time in $N$. Very similar constructions were used in [18, 19, 20].

Having calculated the marginal probabilities, we can also determine the energy of the average coupling vector

$$E = \sum_{\mu=1}^{M} \Theta \left( -x_0^\mu \cdot \sum_{i=1}^{N} \langle J_{i \rightarrow 0} \rangle_i x_i^\mu \right)$$

(12)

and the Bethe entropy

$$S = \sum_{\mu=1}^{M} S_{\mu} - (N - 1) \sum_{i=1}^{N} S_i$$

(13)

characterizing the number of “good” coupling vectors. In the last expression, the site entropy $S_i$ is given by

$$S_i = \sum_{J_i=-1,0,1} P_i(J_{i \rightarrow 0}) \ln P_i(J_{i \rightarrow 0}) ,$$

(14)

and the pattern entropy

$$S_{\mu} = \sum_{\vec{J}} P_{\mu}(\vec{J}) \ln P_{\mu}(\vec{J}) ,$$

$$P_{\mu}(\vec{J}) = \exp \left\{ -\beta \Theta \left[ -x_0^\mu \cdot \sum_{k} J_{k \rightarrow 0} x_k^\mu \right] \right\} \prod_i P_{i \rightarrow \mu}(J_{i \rightarrow 0}) ,$$

(15)

can be calculated in analogy to $\rho_{\mu \rightarrow i}$ via a Gaussian approximation of the sum over $\vec{J}$.

These quantities can be used to fix the free parameters. Imagine, e.g., that we want to achieve some dilution $N_{\text{eff}}(\vec{J})$. Then we can start at high temperature $\beta^{-1}$ and low diluting field $h$, and during the iterative solution of the BP equations we adopt the parameters slowly such that at the end $N_{\text{eff}}(\vec{J})$ takes the desired value, and the entropy $S$ vanishes. This end point corresponds to the ground state at given dilution. In the case that the noise level in the data is known, we can also use this to fix a (possibly pattern- and gene-dependent) temperature right from the beginning.

4. Artificial data

Before applying the algorithm to real biological data, it is useful to check its performance on artificial data. The basic idea is to first generate data by some known network, and then to feed it to our algorithm. The inferred couplings can be compared to the known ones of the generator.
Here we consider a very simple data generator which has the advantage of being analytically tractable with tools from the statistical physics of disordered systems, more precisely with the replica trick. To do so, we still simplify a bit the situation and look only to binary data \( x^\mu_i = \pm 1 \), and to \( N \) inputs \( (i = 1, \ldots, N) \) and one output \( (i = 0) \) which are related to each other by the relation

\[
x^\mu_0 = \text{sign} \left( \sum_{i=1}^N J^0_{i \rightarrow 0} x^\mu_i + \eta^\mu \right),
\]

for all \( \mu = 1, \ldots, M \). The inputs are assumed to be independent and unbiased random numbers. To render the inference task non-trivial, we assume two major differences with respect to the ternary classification done by BP:

- **Heterogeneity of couplings**: The couplings \( J^0_{i \rightarrow 0} \) may take values different from 0, \( \pm 1 \), allowing for weak and strong influences of repressor and activator genes. In this study we use

\[
\rho(J^0_{i \rightarrow 0}) = (1-k_1-k_2)\delta(J^0_{i \rightarrow 0}) + \frac{k_1}{2} [\delta(J^0_{i \rightarrow 0}+1) + \delta(J^0_{i \rightarrow 0}-1)] + \frac{k_2}{2} [\delta(J^0_{i \rightarrow 0}+2) + \delta(J^0_{i \rightarrow 0}-2)].
\]

Generalizations are straightforward. To meet the biological constraint of diluted interactions we assume \( k_1, k_2 \ll 1 \).

- **Noise**: Biological data are noisy. We therefore include white Gaussian noise with

\[
\begin{align*}
\overline{\eta^\mu} & = 0, \\
\overline{\eta^\mu \eta^{\nu'}} & = \gamma N \delta_{\mu,\nu}.
\end{align*}
\]

The scaling of the variance with \( N \) ensures a finite signal-to-noise ratio. For the special case \( \gamma = k_1 + 4k_2 \), the statistical properties of signal and noise are identical.

Obviously, biological data coming out of a full network are correlated even at the input level to gene 0, but we do not consider such correlations here. Since original and inferred couplings take different values, we use the following standard notation for comparing both vectors:

\[
\begin{align*}
J^0_{i \rightarrow 0} = 0 & \quad J_{i \rightarrow 0} = 0 & \text{true negative (TN)} \\
J^0_{i \rightarrow 0} \neq 0 & \quad J_{i \rightarrow 0} = 0 & \text{false negative (FN)} \\
J^0_{i \rightarrow 0} = 0 & \quad J_{i \rightarrow 0} \neq 0 & \text{false positive (FP)} \\
J^0_{i \rightarrow 0} \neq 0 & \quad J_{i \rightarrow 0} \neq 0 & \text{true positive (TP)}
\end{align*}
\]

A major aim in network inference is to predict a fraction of all couplings with high precision, i.e. to have an as high as possible number of TP with a low number of FP. The quality measure we use will be the confrontation of the recall, or sensitivity,

\[
RC = \frac{N_{TP}}{N_{TP} + N_{FN}},
\]

and of the precision, or specificity,

\[
PR = \frac{N_{TP}}{N_{TP} + N_{FP}}.
\]

The recall describes the fraction of all existing non-zero couplings which are predicted by the algorithm, whereas the precision tells us which fraction of all predicted links is actually present in the data generator. Both quantities are in competition: To have a very high precision, only the strongest signals \( P_i(J_{i \rightarrow 0} = 0) \ll 1 \) should be taken into account, but obviously this reduces
the recall. A high recall is achieved by accepting also weaker signals, and obviously the FP rate
will grow accordingly.

The nicest aspect of the simple data generator is that it can be analyzed using the replica
trick, at least in the thermodynamic limit \( N \to \infty \) with \( \alpha = M/N \in \mathcal{O}(1) \) and \( k_1, k_2 = \mathcal{O}(1) \). Details of this analysis are given elsewhere [21], here we only show some results.

Figure 2 shows the performance of BP in the noiseless case for \( k_1 = k_2 = 0.025 \), i.e., only 5% of the couplings in the generator are non-zero. A perfect algorithm would reach recall one with precision one, a completely random algorithm would have precision 5% right from the beginning. Curves in between the two extrema see some of the structure of the generator, but include also false positives. We see that obviously a higher number of patterns improves considerably the performance, but even for \( \alpha = 0.1 \) curves start at precision one. The performance depends on the dilution \( n_{eff} = N_{eff}/N \) of the inferred coupling, a dilution slightly below the one of the generator turns out to be optimal. As a comparison, we have also included the result of a simple pair-based algorithm ranking input genes according to their mutual information with the output, as done in some state-of-the-art approaches to network inference [7, 8, 9, 10]. We see that BP outperforms this approach, mainly because it is able to detect some combinatorial effects which by definition are not seen by a pair-correlation based method.

Figure 3 evaluates the noise influence for the same dilution parameters at \( \alpha = 0.5 \). Curves go from zero noise to \( \gamma = k_1 + 4k_2 \) where input signal and noise have the same variance. The performance of BP obviously goes down with increasing \( \gamma \), but the performance loss is continuous.

To resume this section, we see that BP is able to outperform simpler, pair-based approaches.
Figure 3. Precision versus recall of BP for various noise strengths $\gamma = 0.0, 0.025, 0.05, 0.075, 0.1, 0.125$ (curves from right to left), ranging from no noise to equal signal and noise strengths. Other parameters are $k_1 = k_2 = 0.025$, $n_{eff} = 0.04$ and $\alpha = 0.5$.

For very low pattern number $M$, however, this performance gain is relatively small. Only for intermediate data quantity the strength of BP becomes really relevant.

5. The yeast network

After having analyzed the performance of BP in the case of artificial data, now we discuss its application to real gene-expression data. More specifically we look at genome wide data for baker’s yeast ($\textit{Saccharomyces cerevisae}$) using publicly available data of Gasch, Spellman et al. [22]. Expression levels of 6152 genes have been recorded under 172 environmental conditions (like temperature shock, osmotic pressure, starvation etc.).

First we have preprocessed data: We have eliminated genes which are known to directly respond to external stress [22] since we are interested only in internal regulatory mechanisms. Further on we have filtered out all genes of small variance (we have used three-times the minimum variance), and genes with more than 10 missing data points in the 172 arrays. The resulting 2659 genes are used for inference, whereas the smallest found variance was used as a (slightly pessimistic) estimate of the noise level. We used it to fix the temperature, so only the diluting field remains as a free parameter.

To increase $\alpha$, we did not use all filtered genes as possible input variables, but restricted the search to 460 potential regulators (known TFs, signaling proteins, proteins structurally similar to known regulators etc.) [17]. Further on, to estimate the quality of our prediction, for each of the 2659 output genes we divided the data set ten times randomly into a training set of 142 patterns, and a test set of 30 patterns. BP was run using only the training patterns, and the average coupling vector was used to estimate the generalization error on the test data. Doing so, we had to run BP 26590 times, which took less than two weeks on a single desktop PC.

As an output, we found on average 2.3 regulators per gene. The predictability on the test data was about 76%, which is only slightly better than the one obtained using as a predictor the three most correlated genes (74% correct predictions on average). This result has, however, to be compared to an error bound obtained on the basis of the noise estimate: In particular genes
of expression close to zero have a considerable probability of being measured with opposite sign in the micro-array, leading thus to an \textit{a priori} error in the cost function. Taking the specific values of yeast, we expect about 20\% of all measured expression levels to have the wrong sign compared to the actual mRNA abundance, so the mean predictability cannot grow beyond 80\%.

![Figure 4](image-url) 

**Figure 4.** Fraction of links with common functional annotation, as a function of genes ranked according to their prediction error.

Is there some biological signal in the inferred couplings? To answer this question, we have looked to all those links which have gene-ontology annotations (see http://www.geneontology.org/) for both extremities of the link, and we have determined the fraction of links with at least one common annotation. In figure 4 the results are presented, with the genes ranked according to their predictability (highest rank = smallest prediction error). As a comparison the results for a null model (randomly rewired graphs having the same in- and out-degrees as the inferred one) are depicted. Symbols and bars represent averages and variances of the null model. It is obvious that the BP results show a significant enrichment in common functional annotations, with a strong correlation to the quality of the prediction error. No such signal is detected if we use the three most correlated input genes as predictors.

Last but not least, figures 5 and 6 show an example of combinatorial control. The upper part of figure 5 shows the expression of gene YGL001 together with its prediction by the BP selected input regulators, the left bars depicts the training, the right ones the test set. The lower bars are the three most important input genes according to BP. Note that some of the cases where the first input gene has an opposite sign compared to the output gene are cured by the two other genes, which gives a clear illustration of combinatorial control. Figure 6 shows the analogous result for the three most correlated genes. The first input here is identical to the one found by BP, but its errors are not corrected by the other inputs. In fact we find the prediction on the test set to be much better for BP than for pair correlations.

6. Conclusion and outlook
In this article, we have introduced, analyzed and applied a message-passing algorithm for the inference of gene interactions from genome-wide expression experiments. With the aim
Figure 5. Measured vs. predicted gene expression with belief propagation: The first bar is the measured gene, divided in training and test set. The second bar is the prediction using BP, the following three rows are the most relevant input genes according to BP. Repression is denoted in blue, activation in red. Patterns are ordered according to the expression level of the output gene.

Figure 6. Measured vs. predicted gene expression by the most correlated genes: The first bar is the measured gene, divided in training and test set, the second bar is the prediction by the three most correlated genes, the following three bars are the input genes of highest correlation to the output in the training set.

to infer a sparse, directed network showing combinatorial control we have set up a minimal model classifying regulators into three classes: repressors, activators or non-regulators. Even if simplified compared to biological reality, this simple model is expected to reflect part of the relevant biological processes.

In the case of artificial data we have shown that the algorithm predicts some true positive links even in the case of very few input patterns. The performance increases if more patterns are present. We have also seen that BP outperforms simpler pair-correlation based approaches. The advantage of BP was small for few data, and big for an intermediate number of data. In the limit of infinitely many data both algorithms are expected to perform well.

In the case of biological data, more precisely yeast expression profiles, a small but systematic advantage of BP compared to correlations was observed. This suggests that we are still in the low-data regime, and more data would be needed to profit from the high potential of message passing techniques. However, we have seen that genes resulting in a smaller than average prediction error show strongly enriched common functional annotations between regulators and regulated genes. We therefore expect that more and less noisy data lead to a more pronounced
difference between BP and traditional pair-correlation based tools.

One of the weak points in the derivation of the algorithm is that we neglected correlations between input genes. It is known that these correlations are strong in biological data, so it appears to be important to study systematically the influence of correlations on the performance of BP. This will be done in a future work.

Further on, the fact that only few data are available requires the integration of further biological knowledge. We already used a precompiled list of potential regulators (instead of all genes), but it could be highly interesting to include also sequence information on putative binding sites.

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