

Targeting c-Myc-activated genes with a correlation method: Detection of global changes in large gene expression network dynamics

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This work studies the dynamics of a gene expression time series network. The network, which is obtained from the correlation of gene expressions, exhibits global dynamic properties that emerge after a cell state perturbation. The main features of this network appear to be more robust when compared with those obtained with a network obtained from a linear Markov model. In particular, the network properties strongly depend on the exact time sequence relationships between genes and are destroyed by random temporal data shuffling. We discuss in detail the problem of finding targets of the *c-myc* protooncogene, which encodes a transcriptional regulator whose inappropriate expression has been correlated with a wide array of malignancies. The data used for network construction are a time series of gene expression, collected by microarray analysis of a rat fibroblast cell line expressing a conditional Myc-estrogen receptor oncoprotein. We show that the correlation-based model can establish a clear relationship between network structure and the cascade of c-myc-activated genes.

complex systems | time series | gene interaction

The availability in modern molecular biology of methods capable of measuring the activity of thousands of genes at the same time poses the challenge of analysis and modeling of complex biological networks with thousands of units. Microarray technology is producing data on the activity of significant portions of the genome in a wide variety of cells and organisms up to the level of the entire human genome. Several techniques have been proposed to analyze the high dimensional data resulting from these experiments. Artificial neural networks, phylogenetic-type trees, clustering algorithms, and kernel methods are just a few examples (1–6).

Complex network theory has been used to characterize topological features of many biological systems such as metabolic pathways, protein–protein interactions, and neural networks (7, 8). The application of network theory to gene expression data has been not fully investigated, particularly regarding the time-dependent relationships between genes occurring while their expression level changes.

One of the key points of the network approach is the definition of the links between its elements (nodes), namely, the gene interactions from which all of the network properties are obtained. Recently, several methods for links assessment have been proposed, such as linear Markov model (LMM)-based methods (9, 10) or correlation-based methods (11–13). We choose to define links on the basis of the time correlation properties of gene expression measurements.

In this article, we show that correlation properties of gene expression time series measurements reflect very broad changes in genomic activity. The problem that we address is characterizing the gene transregulation cascade in response to c-Myc protooncogene activation. *C-myc* encodes a transcriptional reg-

ulator whose inappropriate expression is correlated with a wide array of malignancies. At the cellular level c-Myc activity has been linked with cell division, accumulation of mass, differentiation, and programmed cell death. Although the positive influence of c-Myc on proliferation has been appreciated for a long time, the molecular mechanisms by which these end points are achieved are not well understood. It is now clear that Myc can directly influence the expression of thousands of genes with diverse functions. A significant challenge is to integrate this wealth of information into mechanistic models that explain the biological functions of c-Myc. This endeavor has been greatly complicated not only by the large number of targets, but also by the weak transcriptional effects exerted by c-Myc. Thus, the biologically relevant downstream effectors remain to be comprehensively delineated.

The correlation method is more sensitive to the temporal structure of the data than LMM and leads to biologically relevant gene identification that is not obtained by either Markov modeling or significance analysis based only on ANOVA.

Methods

Gene Expression Time Series. Two data sets of gene expression were obtained from a set of microarray experiments using genetically engineered rat cell lines. As described (ref. 14 and references therein), parental Rat-1 fibroblasts were modified by homologous recombination to knock out both copies of the *c-myc* gene (*c-myc*^{-/-} cells). This cell line was subsequently reconstituted with a cDNA encoding a fusion protein of c-Myc and the human estrogen receptor (MycER). The fusion protein is synthesized continuously in the cells, but is biologically inactive in the absence of a specific ligand, 4-hydroxy tamoxifen. Binding of tamoxifen to the estrogen receptor domain elicits a conformational change that allows the fusion protein to migrate to the nucleus and act as a transcription factor. A large volume of data from several laboratories indicates that the biological activities of native c-Myc protein and the MycER fusion protein are similar, if not identical. Randomly cycling, exponential-phase cultures were used, and conditions were developed such that cells experienced a constant environment and were in a balanced, steady state of growth for significant periods of time. Two data sets were obtained. The first data set (*N* data set) contains the gene expression data of the *c-myc*^{-/-} MycER cell line treated with vehicle (ethanol) only. The second data set (*T* data set) contains the gene expression data collected after the addition of tamoxifen. Samples were harvested at five time points after the addition of tamoxifen to the culture medium: 1, 2, 4, 8, and 16 h. The entire experiment was repeated on three separate occasions,

Abbreviation: LMM, linear Markov model.

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Table 2. c-Myc target genes extracted from the selected 1,191 probe sets

GenBank	Name	Description
D13921	Acat1	Acetyl-coenzyme A acetyltransferase 1
J02752	Acox1	Acyl-coA oxidase
AA799466	Ak2	Adenylate kinase 2
M73714	Aldh3a2	Aldehyde dehydrogenase family 3, subfamily A2
M60322	Aldr1	Aldehyde reductase 1
AI177096	Aprt	Adenine phosphoribosyl transferase transferase (APRT)
U07201	Asns	Asparagine synthetase
U00926	Atp5d	ATP synthase, F1 complex, delta subunit
At4g36870	Blh2	BEL1-like homeobox 2 protein
M81681	Blvra	Biliverdin reductase A
AA859938	Bnip31	BCL2/adenovirus E1B 19-kDa-interacting protein 3-like
AI178135	C1qbp	Complement component 1, q subcomponent binding protein
L24907	Camk1	Regulator of G-protein signaling 19
U53858	Capn1	Calpain 1
U53859	Capns1	Calpain, small subunit 1
D89069	Cbr1	Carbonyl reductase 1
AA891207	Cd36l2	CD36 antigen (collagen type I receptor, thrombospondin receptor)-like 2
D26564	Cdc37	Cell division cycle 37 homolog
L11007	Cdk4	Cyclin-dependent kinase 4
AB009999	Cds1	CDP-diacylglycerol synthase
U66470	Cgref1	Cell growth regulator with EF hand domain 1
M15882	Clta	Clathrin, light polypeptide (Lca)
D28557	Csda	Cold shock domain protein A
AI008888	Cstb	Cystatin B
AJ000485	Cyln2	Cytoplasmic linker 2
U95727	Dnaja2	Dnaj (Hsp40) homolog, subfamily A, member 2
U08976	Ech1	Enoyl coenzyme A hydratase 1
D38056	Efna1	Ephrin A1
U19516	Eif2b5	Initiation factor eIF-2Be
X03362	ErbB2	v-erb-b2 oncogene homolog 2
U36482	Erp29	Endoplasmic reticulum protein 29
J04473	Fh1	Fumarate hydratase 1
AI231547	Fkbp4	FK506 binding protein 4 (59 kDa)
M81225	Fnta	Farnesyltransferase, CAAX box, α
AI136396	Fntb	Farnesyltransferase β subunit
AA891857	Fxc1	Fractured callus expressed transcript 1
AA892649	Gabarap	γ -Aminobutyric acid receptor associated protein
J03588	Gamt	Guanidinoacetate methyltransferase
D30735	Gfer	Growth factor, erv1-like
U38379	Ggh	γ -Glutamyl hydrolase
AA944423	Gm130	cis-Golgi matrix protein GM130
AA799779	Gnpat	Acyl-CoA: dihydroxyacetone phosphate acyltransferase
U62940	Grpel1	GrpE-like 1, mitochondrial
X04229	Gstm1	Glutathione S-transferase, μ 1

Table 2. (continued)

GenBank	Name	Description
AB008807	Gsto1	Glutathione S-transferase ω 1
D16478	Hadha	Hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-coenzyme A hydratase (trifunctional protein), α subunit
AA892036	Hdac6	Histone deacetylase 6
X52625	Hmgcs1	3-Hydroxy-3-methylglutaryl-coenzyme A synthase 1
D14048	Hnrpu	System N1 Na ⁺ and H ⁺ -coupled glutamine transporter
S57565	Hrh2	Histamine receptor H
AA957923	Mcpt2	Mast cell protease 2
U62635	Mrp123	Mitochondrial ribosomal protein L23
AF104399	Msg1	Melanocyte-specific gene 1 protein
X93495	Mtap6	Microtubule-associated protein 6
M55017	Ncl	Nucleolin
AF045564	Ndr4	N-myc downstream regulated
AA874794	Ngfrap1	Nerve growth factor receptor associated protein 1
AA998882	Nopp140	Nucleolar phosphoprotein p130
J04943	Npm1	Nucleophosmin 1
M25804	Nr1d1	Nuclear receptor subfamily 1, group D, member 1
AB015724	Nrbf1	Nuclear receptor binding factor 1
AA800679	Ns	Nucleostemin
D13309	Nsep1	Nuclease sensitive element binding protein 1
X82445	Nudc	Nuclear distribution gene C homolog
U03416	Olfm1	Olfactomedin-related ER localized protein
U26541	Pdap1	PDGFA-associated protein 1
M80601	Pdcd2	Programmed cell death 2
S82627	Pem	Placentae and embryos oncofetal gene
AI169417	Pgam1	Phosphoglycerate mutase 1
AA998446	Pitpnb	Phosphotidylinositol transfer protein, β
X71898	Plaur	Plasminogen activator, urokinase receptor
L25331	Plod	Procollagen-lysine hydroxylase
S55427	Pmp22	Peripheral myelin protein 22
AJ222691	Pold1	DNA polymerase delta, catalytic subunit
AB017711	Polr2f	Polymerase II
Z71925	Polr2g	RNA polymerase II polypeptide G
AA892298	Ppil3	Peptidylprolyl isomerase (cyclophilin)-like 3
Y17295	Prdx6	Peroxiredoxin 6
D85435	Prkcdp	PKC-delta binding protein
D26180	Prkcl1	Protein kinase C-like 1
AA891871	Prpsap1	Phosphoribosylpyrophosphate synthetase-associated protein
D10756	Psma5	Proteasome subunit, α type 5
D10755	Psma6	Proteasome subunit, α type 6
U03388	Ptgs1	Prostaglandin-endoperoxide synthase 1
L27843	Ptp4a1	Protein tyrosine phosphatase 4a1
U53475	Rab8b	GTPase Rab8b
AA956332	Rabep1	Rabaptin 5
L19699	Ralb	v-ral oncogene homolog B
U82591	Rcl	Chromosome 6 open reading frame 108

dynamics should be significantly affected by a random shuffling in time.

Results

When c-Myc is activated by tamoxifen stimulation, the activity profile of the probe sets clearly changes into a strongly correlated regime. These findings are reflected in the histograms of the correlation coefficients for the *N* and *T* data sets (Fig. 1) and in

the main parameters of the connectivity distributions obtained from the corresponding adjacency matrices (Table 1). For the *T* data set, the number of coefficients close to +1 or -1 increases significantly. This finding is an indication that many of the 1,191 genes that were affected mostly by tamoxifen stimulation in their expression levels over time became either strongly correlated or anticorrelated.

Both networks appear to be highly clustered (Table 1), as compared with a random network with the same number of

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