

A computer model for the kinetics of chromatin modifications at the immediate early gene promoter – brief communication

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Abstract

Combinatorial histone protein modification code, namely phosphorylation, acetylation, ubiquitination, or methylation, indicates to the gene expression machinery what specific transcription events are to occur: activation, repression, or silencing of the gene or locus in question. Many laboratory experiments address this issue using a variety of systems, such as global chromatin modifications in the human immunoglobulin locus. In this study, we investigate a small-scale histone modification event in the context of immediate early gene (IEG) regulation.

Immediate early gene activation upon mitogenic activation is a critical step in the regulation of cellular growth, and involves elaborate control and modification of the local chromatin environment. In this conceptual model, we have investigated the histone modification events following the mitogen-activated protein kinase (MAPK) / extracellular signal-regulated kinase (ERK) pathway activation upon growth factor stimulation. Gepasi simulation environment 3.30 version has been used to test this conceptual model. Since experimental dissection of histone modifications and kinetics upon MAPK activation is technically challenging, computer models of such modifications possess immeasurable predictive power in targeted experimental design in the future.

Introduction

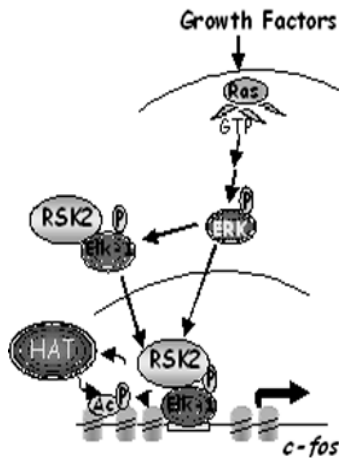


Figure 1. Cartoon representation of IEG regulation in response to growth factors.

Cells respond to extracellular stimuli by activation of various signaling pathways that result in proliferation, survival, differentiation, or apoptosis. Growth factors, such as Epidermal Growth Factor (EGF) elicit cellular responses through activation of the Ras / MAPK pathway and upregulation of immediate-early genes (IEG) [1,23,24,28]. The IEG promoters contain serum response elements (SREs) that bind ETS domain transcription factors, such as Elk-1, which represent one level of regulation for gene activation in response to MAPK signaling [10,16,17,25].

Another group of downstream targets of the MAPK pathway is the MAPKAP kinase family, in particular RSK2 [3,21,22,24]. RSK kinases have recently been identified as histone H3 kinase [6,7,28,31]. Stimulation of mammalian cells by epidermal growth factor (EGF) results in rapid and sequential phosphorylation and acetylation of histone H3 on the immediate-early gene *c-fos* promoter, and both of these histone modifications are known to be positive regulators of gene activation [6,7] (see Figure 1). Although a mechanistic link between these two enzymatic reactions (phosphorylation and acetylation) is further suggested by the finding that, in vitro, several transcription-associated histone acetyltransferases (HATs) display strong preferences for histone H3 phosphorylated at Serine 10 over the unmodified form as

substrate [6,18], the sequence of events from MAPK activation to *c-fos* transcription is still inaccessible using experimental techniques.

Computer simulations or mathematical models of different aspects of MAPK signal transduction pathway has been performed recently [1,14,26], however none of these models have integrated transcriptional regulation through chromatin to the upstream pathway activation. In order to address chromatin modification events, we have established a computer model using the GEPASI simulation environment developed by Pedro Mendes initially in C programming language to simulate metabolic reactions and integrating ODEs (ordinary differential equations) [19,20]. The enzymes, such as kinases or histone acetyl transferases, are represented by classical Michaelis-Menten kinetics [1,14,19,20,26].

Materials and Methods

Biochemical Parameters

Biochemical parameters for the base chromatin modification model proposed have been obtained from literature records of wet-lab experiments wherever available, and from previous computational estimates otherwise (see Table 1).

Table 1. A summary of critical quantities for the base model of histone modifications.

Metabolite	Initial Conc.	K_M (uM)	k_{cat} (sec ⁻¹)	REF
ERK	750,000 molecules	20000 (for RSK2.Elk)	5 (for RSK2.Elk)	[1,10]
RSK2.Elk.HAT	200,000 molecules			[3]
RNA pol II	100,000 molecules	50 (for Ac-P histone promoter) 100 (for P-histone promoter)	4.5×10^{-3} (for Ac-P-histone promoter) 2.5×10^{-3} (for P-histone promoter)	[2,5,11,12] [2,5,11,12]
DNA.histone	100,000 molecules			A
Active HAT	0	28 (for phosphorylated histone) 280 (for unmodified histone)	0.28 (for phosphorylated histone) 0.34 (for unmodified histone)	[6,9,17,18,22,27] [6,9,17,18,22,27]
RSK2.Elk.P	0	10000 (for unmodified histone)	5 (for unmodified histone)	[4,8,13,18,22]

a. Computationally determined.

Computer simulations

The latest version of the standard biochemical kinetics simulation software package, GEPASI 3.30 (Mendes 1997;Mendes 1998), has been used to develop a computer model of the MAPK-induced immediate early gene activation. The pathway was based on an EGF activated signalling pathway and parameters therein (Aksan and Kurnaz, 2002, <http://www.chemphys.boun.edu.tr/biochem/index.html>), any additional

parameters specifically for this model have been noted in Table 1. The graphs were generated using GnuPlot and GEPASI 3.30, showing transient states during a time course of 10 minutes.

Results and Discussion

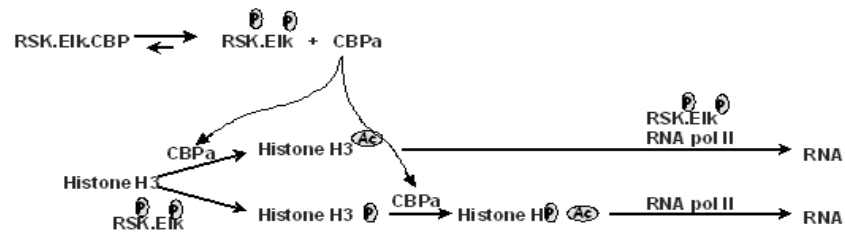
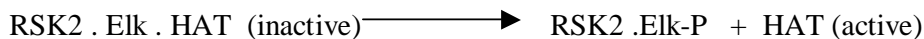


Figure 2. The outline of the reactions used to build the Gepasi model in this study.

Histone modifications based on a pre-formed RSK2-Elk-1-HAT complex

Our model (see Figure 2) for chromatin modifications were based on the assumption that RSK2 and Elk-1 were always in complex, regardless of growth factor stimulation [15, Aksan and Sharrocks, unpublished experimental observations]. This complex was inactive and unable to bind the *c-fos* promoter (DNA) unless stimulated by a growth factor.

The histone acetyl transferase CBP (CREB-binding protein) was experimentally observed to be in complex with RSK2 in an inactive state (unable to acetylate histones) [17]. Upon phosphorylation of RSK2 by ERK MAPK, CBP dissociates from the complex, and is thereby activated. We have incorporated this observation into our model as follows:



Phosphorylated RSK2, Elk-P complex was then allowed to bind the promoter, phosphorylate neighboring histones, and recruit RNA polymerase II to the initiation site. The active HAT would in parallel acetylate either unmodified histones or phosphorylated histones in the promoter region, with different kinetics (see Table 1).

When this model was stimulated with 100 nm EGF (epidermal growth factor, standard stimulating conditions), we could achieve the immediate activation of ERK MAPK within minutes of stimulation (Figure 3), consistent with experimental evidence [10,17,25,29,30].

When transient concentrations of RSK2 · Elk · HAT complex, RSK2 · Elk-P and active HAT were plotted, the accumulation of active HAT (CBP) was seen to be extremely rapid, with maximal concentration achieved within minutes (almost instantaneously as ERK is activated, Figure 4).

Histone modifications, namely acetylation, phosphorylation and phosphor-acetylation, were then monitored (Figure 5). The surprising result was that nearly no acetyl-

histone accumulated using in the model the kinetic constants reported experimentally (see Table 1, and Figure 5, DHA). This is presumable because acetyl-histone H3 rapidly gets di-modified. Phosphorylation occurred with immediate nevertheless transient kinetics, and the di-modified histone containing promoter had a delayed but more sustained kinetics than phospho-histone promoter. This is a novel and important finding, since such early stages in growth factor induction kinetics could not previously be addressed and experiments failed to observe this initial and rapid histone phosphorylation event [only around 5 % was reported, 6,9,28].

Under the conditions used in the model, RNA synthesis was visible from as early as 1 minute after stimulation (the sensitivity of experimentation was only sufficient to show *c-fos* transcription within 5 minutes) [5,11].

The proposed model with a pre-formed RSK2, Elk-1 and HAT complex rendering promoters in a 'poised' state appears to be in agreement with published data, and the rapid activation upon stimulation is a critical result that supports earlier suggestions that phosphorylation precedes acetylation, and transcription follows dimodification of histones [6]. Such a poised state would also allow for rapid shut-off of the transcriptional events to the basal level, a crucial requirement of the activation / inactivation cycle of immediate-early gene promoters. The inactivating mechanisms are not yet clear, and extensions of the model presented here can aid researchers in designing critical experiments.

In spite of the recent trend in computer modeling, we are still at a very early stage, but with increasing accumulation of biochemical data the simulations will inevitably become more reliable and more useful in testing alternative models for *in vivo* feasibility, and gain more predictive power.

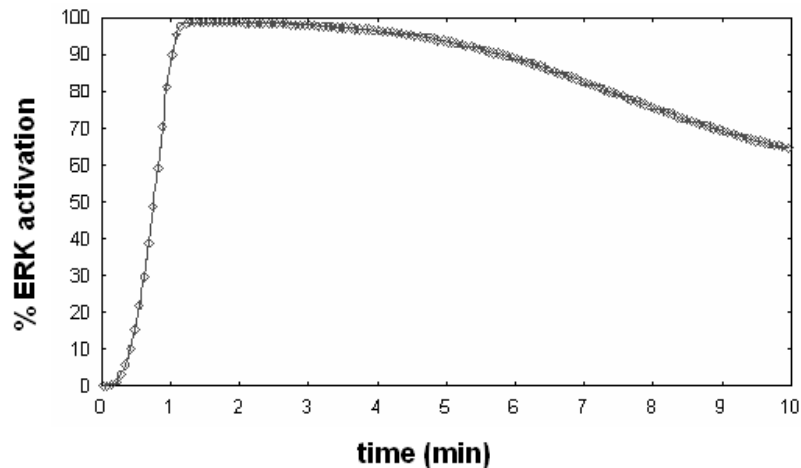


Figure 3. ERK MAPK activation kinetics in response to growth factor.

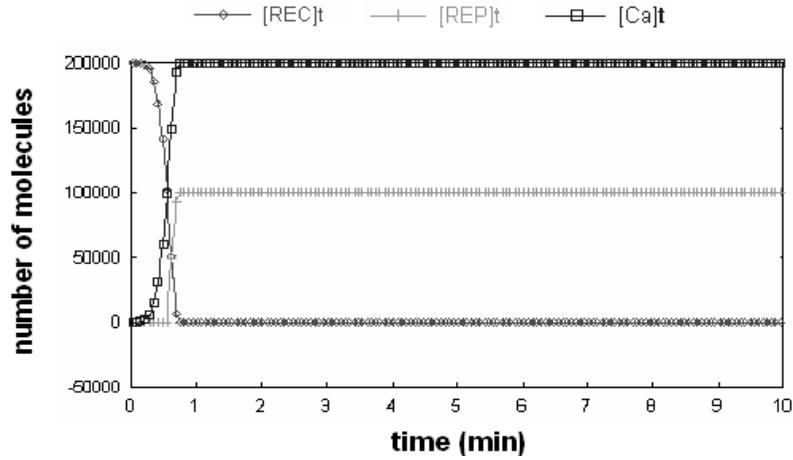


Figure 4. Time course of RSK2, Elk and HAT CBP complex interactions. Initially all proteins are found in the same complex in an inactive state (REC). As RSK2 . Elk-1 gets phosphorylated (REP), HAT CBP dissociates and is activated (C_a).

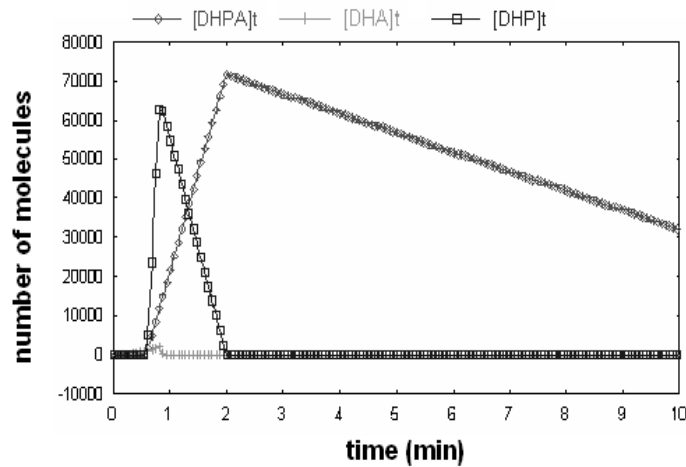


Figure 5. Histone modification kinetics. DHPA: phosphoacetyl-histone containing promoter, DHA: acetyl-histone containing promoter, and DHP: phosphor-histone containing promoter.

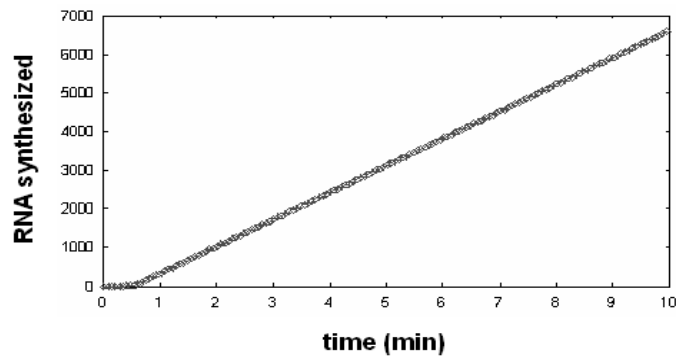


Figure 6. RNA synthesis immediately after growth factor stimulation.

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