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Hierarchical cooperativity mediated by chromatin remodeling; the model of the MMTV transcription regulation

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ABSTRACT

Chromatin remodeling machineries are abundant and diverse in eukaryotic cells but their importance in gene expression remains unclear. Although the influence of nucleosome position on the regulation of gene expression is generally envisioned under the equilibrium perspective, it is proposed that given the ATP-dependence of chromatin remodeling enzymes, certain mechanisms necessitate non-equilibrium treatments. In particular, examination of the celebrated chromatin remodeling system of the mouse mammary tumor virus, in which the binding of transcription factors opens the way to other ones, reveals that breaking equilibrium offers a subtle mode of transcription factor cooperativity, avoids molecular trapping phenomena and allows to reconcile previously conflicting experimental data. The mechanism proposed here provides a control lever of promoter sensitivity and responsiveness, increasing the discernment of gene expression.

1. Introduction

The specificity and intensity of gene expression is governed by interactions between regulatory DNA sequences (*cis*-regulators) and various *trans*-acting factors (transcription factor proteins (TFs) and non-coding RNAs). The occupation of a gene promoter by these *trans*-regulators involves both micro-reversible and micro-irreversible steps. Micro-reversible binding processes can lead to sigmoidal concentration-dependent response through classical multimeric cooperativity (Bolouri and Davidson, 2002; Michel, 2010). The role of nucleosomes has also been examined from the micro-reversible perspective (Dodd et al., 2007; Segal and Widom, 2009; Mirny, 2010). The rapid equilibration of these thermally driven phenomena, relatively to the slow changes of cellular components, simplifies the definition of the input functions used in gene network modeling (Bintu et al., 2005; Michel, 2010). But promoter occupancy also involves some micro-irreversible transitions such as chromatin remodeling and active dissociation processes. Precisely, it is proposed in the present study that inserting micro-irreversible steps in the process of promoter saturation opens additional possibilities of potent cooperativity. A single example has been selected because it remarkably illustrates how micro-irreversible transitions can generate a refined discernment in gene expression. In this example, the micro-irreversible step corresponds to the energy-dependent phenomenon of chromatin remodeling, in which the position of DNA around nucleosomes is modified. By this way, upon binding to DNA, a first TF directs the accessibility to DNA of other ones. This mechanical activity allows (**i**) cooperativity between non-physically interacting TFs and (**ii**) constitutively expressed TFs to participate to conditional induction. One of the most celebrated chromatin remodeling system is provided by the thoroughly documented Mouse Mammary Tumor Virus (MMTV) promoter.

2. Breaking hierarchical equilibrium is necessary to maintain molecular interaction dynamics

The assembly of macromolecular complexes generally proceeds in a hierarchical manner in the cell. For example, a component C, which cannot bind to the isolated components A and B, can bind to a pre-associated complex AB. Hierarchical binding chains such as $A+B \leftrightarrows AB, +C \leftrightarrows ABC, +D \leftrightarrows ABCD\ldots$ etc. are often involved in the building of multi-molecular complexes, but are less compatible with the dynamic and reactive behaviors of soluble components. Indeed, in equilibrium conditions, the chain written above would lead to the trapping of the early components in the complexes as long as the late components are present. This phenomenon can exist for TF binding to gene promoters. It can hold, for example, for the hierarchy between the successive binding steps observed in equilibrium conditions between the TodT TFs and the series of TodT-binding sites juxtaposed in the *Tod* gene promoter and that has been proposed to be mediated by DNA conformation changes (Lacal et al., 2008). Beside this

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puzzling situation of equilibrium allostery, the hierarchical binding of transcription factors in equilibrium conditions is also possible in the case of the large eukaryotic preinitiation complexes made of the so-called general transcription factors GTFs (Michel, 2010). But hierarchical relationships have also been reported for non-interacting isolated TFs in absence of any trapping phenomenon. To allow reconciling hierarchical binding and absence of trapping, one should postulate the possibility to break equilibrium. This situation is well illustrated by the case of the occupation of the MMTV promoter involving micro-irreversible processes, thoroughly documented but not yet clearly understood. In Section 3, this system will be analyzed under the classical equilibrium assumption. Then, in Section 4, a non-equilibrium scheme will be proposed based on hypotheses derived from MMTV experimental data, in which the equilibrium-breaking machines are the ATP-dependent SWI/SNF chromatin remodeling enzymes.

3. Data obtained for the MMTV promoter are irreconcilable from the time-reversible perspective

A central piece of data about MMTV expression is the role of nucleosomes in the mutual influence between the glucocorticoid receptor (GR) and a group of TFs (NF1/Oct). Although the activation of MMTV by GR and NF1/Oct1 seemed clear in the initial reports, discrepancies appeared in following studies. The basis of glucocorticoid hormone-induced MMTV regulation is that GR has an initiating role, triggered upon hormone binding (stress hormone corticol or corticosterone) and subsequently amplified by NF1 and Oct1. This sequential action is dependent on the position of nucleosomes on DNA, since it is not observed with naked DNA (Richard-Foy and Hager, 1987; Archer et al., 1992; Chávez and Beato, 1997). The repositioning of nucleosomes triggered by GR, probed by nuclease or chemical mapping, leads to the exposure of the NF1 and Oct1 binding sites and is mediated by SWI-SNF ATPases (Fryer and Archer, 1998). The different roles of GR and NF1 in initiating and amplifying transcription, respectively, are explained by their differential mode of interaction with chromatin: GR can bind to DNA wrapped around nucleosomes, contrary to NF1, which requires a fully accessible double helix (Eisfeld et al., 1997). This DNA-binding hierarchy, first of hormone-bound GR and then of NF1/Oct, offers a powerful opportunity of cooperativity illustrated by the comparison between Fig. 2a and 2b. GR is inducible but not very potent contrary to the couple NF1/Oct. As NF1/Oct can access DNA only upon binding of GR, the promoter activity can become sigmoidal (Fig. 2b) particularly when NF1/Oct is transcriptionally more potent than GR. Sigmoidal responses are generally due to decreased responsiveness to low signals. This is the case for the MMTV promoter in which TFs are prevented to bind at low concentration.

But this elegant mechanism has then been clouded in the following reports, which introduced new actors and revised the hierarchy of binding of GR and NF1. In sharp contrast with the earlier articles, NF1 and Oct1 binding sites have been shown to preset chromatin prior to GR binding (Belikov et al., 2004). The picture is thus more complex than supposed previously and the mutual influence between GR and NF1 for binding to the MMTV promoter is now described as dualistic (Hebbar and Archer, 2007), blurring the logic of this system. It will be shown that the cooperative relationships between GR and NF1, which are unclear when examined only from a time-reversible perspective, can be usefully reconsidered from a non-equilibrium perspective (Section 5), but the outcomes of equilibrium modeling is first examined below for comparison.

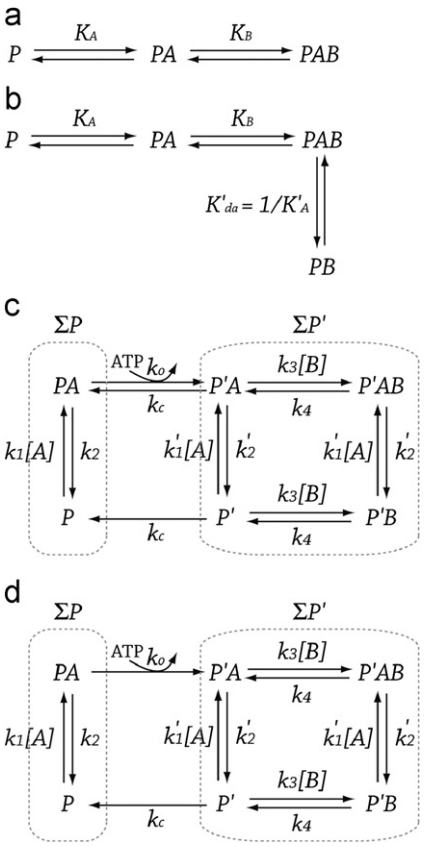

Fig. 1. Different models to explain the hierarchical occupation of the MMTV promoter (P) by GR (named A) and NF1 (named B). The schemes **a** and **b** comply with the principle of microscopic reversibility but not the **c** and **d** ones. In the scheme **c**, k_0 is the rate of chromatin opening driven by SWI/SNF ATPases and k_c is the rate of chromatin closing, driven by stabilization of DNA bending. B cannot bind to P because of inappropriate nucleosome positioning, while A can bind to both P and P' with different constants. In the scheme **d**, chromatin closing can occur only when the promoter is free of any TF.

4. Equilibrium modeling of cooperative MMTV promoter occupancy

In the simplest hierarchical modeling scheme assuming microreversibility (Fig. 1a), MMTV transcription is stimulated by two groups of transcription factors GR (named A) and NF1/Oct (named B). A and B bind to the MMTV promoter (P) through their DNA-binding domain (DBD), in a hierarchical manner, but once bound to DNA, they are supposed stimulate transcription in an independent and additive manner, through their activation domain (TAD). In these conditions, the fractional activity (F) ranging from 0 to 1, of the MMTV promoter, is described in Eq. (1). In this equation, k_A and k_B are the maximal frequencies at which A and B, when bound to DNA, recruit transcription machineries, thereby initiating successive rounds of transcription. The maximal frequencies k_A and k_B should be weighted by the probabilities of presence of A and B in the promoter, written $p(A)$ and $p(B)$, respectively (with small letters p to not be confused with the promoter P):

$$F = \frac{p(A)k_A + p(B)k_B}{k_A + k_B} \tag{1}$$

The probabilities $p(A)$ and $p(B)$, equivalent to fractional occupation times, can be formulated through an Adair approach as the ratio of occupied over total binding sites, which can be expressed as concentration variables in ergodic conditions, by enumerating the possible promoter states:

$$p(A) = ([PA] + [PAB])/([P_0] + [PA] + [PAB]) \tag{2a}$$