Genome Biology

RESEARCH HIGHLIGHT

Fixated on fixation: using ChIP to interrogate the dynamics of chromatin interactions

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Abstract

A new study exploits the time-dependence of formaldehyde cross-linking in the commonly used chromatin immunoprecipitation (ChIP) assay to infer the on and off rates for site-specific chromatin interactions.

Introduction

Efficient control of gene expression is crucial in nearly all biological processes. This control is exerted, among other things, by physical interactions between DNA regulatory regions and proteins, reading and executing the DNA-encoded instructions. Studies of the spatial organization of DNA-binding proteins (DBPs) across species, tissues, external conditions and perturbations have proven invaluable in elucidating the regulatory mechanisms underlying transcription. Nonetheless, binding of protein and DNA is a dynamic process, in which the two associate and dissociate at certain rates, commonly referred to as the on and off rates of the reaction. Thus, even though determining average occupancies of a DBP across a population of cells is highly informative, knowing the exact rates is crucial for modeling the system and studying its dynamics. In addition, different combinations of association and dissociation rates could have different downstream effects, even if they result in the same average occupancy across cells. For example, fractional occupancy of a DBP at a specific site can indicate strong binding of the factor to this DNA location in some of the cells or, alternatively, that the factor is transiently bound in many cells. These two scenarios might have important downstream implications for the resulting expression of the regulated gene.

Measuring protein-DNA interactions using chromatin immunoprecipitation

The most widespread experimental method for determining where chromatin-binding factors interact with DNA sequences is the chromatin immunoprecipitation (ChIP) assay. In this method, cellular constituents are cross-linked by means of either UV light or, more commonly, formaldehyde to stabilize protein-DNA interactions. Next, the isolated chromatin is fragmented, and protein-DNA complexes are recovered by immunoprecipitation using an antibody that detects the protein of interest. DNA sequences bound to the factor are then interrogated using various techniques, such as PCR, hybridization and, more recently, DNA sequencing [1]. Although this standard ChIP protocol is useful in revealing the relatively specific location of protein binding, it is limited in its ability to provide kinetic information.

In a recently published study, Poorey *et al.* [2] applied chemical reaction rate theory to model what happens during a ChIP experiment and have consequently adjusted the standard ChIP assay described above to allow the extraction of kinetic information. In the modified protocol, they perform several repetitions of the basic ChIP assay, with cross-linking times varying from fractions of a second to 30 minutes and binding assayed by quantitative PCR. The rationale underlying the investigation is that the ChIP signal observed at the end of the experiment represents an integration of the signal throughout the entire fixation period. When formaldehyde is added to the cell constituents, assuming that cross-linking occurs rapidly, it captures the existing *in vivo* occupancy at the time of addition at a rapid rate driven by cross-linking kinetics. From this time onwards, cross-linking ensures that bound molecules can no longer dissociate from DNA, effectively eliminating the off rate. Throughout the fixation time, protein molecules continue to bind to DNA, and these binding events are captured (cross-linked) owing to the presence of formaldehyde. Thus, further increases in signal are governed by the on rate. Fitting the model to several points with

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various fixation times, and constraining the fit by simultaneously fitting two sets of experiments with different concentrations of the binding proteins, allows the extraction of the different kinetic parameters (Figure 1). The authors name this approach the cross-linking kinetic (CLK) method.

To test the CLK method experimentally, the authors applied it to three transcription factors (TFs) in the budding yeast *Saccharomyces cerevisiae*, spanning a range of interaction kinetics: Gal4 interacting with the *GAL3* promoter, Ace1-green fluorescent protein (GFP) interacting with the *CUP1* gene array and LacI-GFP interacting with an array of Lac operators. In all cases, they observed a biphasic behavior, with short fixation times leading to a fast and dramatic increase in ChIP signal, and longer incubation times resulting in a more gradual increase, as predicted by the model. They were able to extract half-life ($t_{1/2}$) times ranging from 11 seconds (Ace1-GFP) to 10 and 20 minutes (Gal4 and LacI-GFP, respectively), suggesting that the method is suitable for a large dynamic range of interactions. The $t_{1/2}$ values for the GFP-bound TFs were also tested by an independent method, fluorescence recovery after photobleaching (FRAP), which yielded a reasonably good agreement with the CLK results.

Using CLK to examine the dynamics of TBP-promoter associations

The authors applied the CLK method to investigate the interaction of the TATA-binding protein (TBP) with seven different promoters driven by different RNA polymerases (pol I, pol II and pol III) that possess diverse transcriptional activities and then deduced both the steady-state occupancies and interaction half-lives. They found low occupancies for all examined promoters, suggesting that stable TBP-promoter complexes *in vivo* are infrequent and that most promoters are not occupied at steady state. Owing to the inherent limitations of the ChIP method, such as the low efficiency of cross-linking and immunoprecipitation, these values do not represent the actual fractional occupancies in the population, but they can provide rank-ordered estimates of fractional occupancy. Moreover, the authors found that TBP-promoter interactions varied dramatically for the different promoters, with $t_{1/2}$ values ranging from 1 to approximately 30 minutes. It is not known what causes these different dynamics, and revealing the factors that stabilize or destabilize TBP interactions with promoters and understanding their quantitative effect on the kinetic parameters are important avenues for future research.

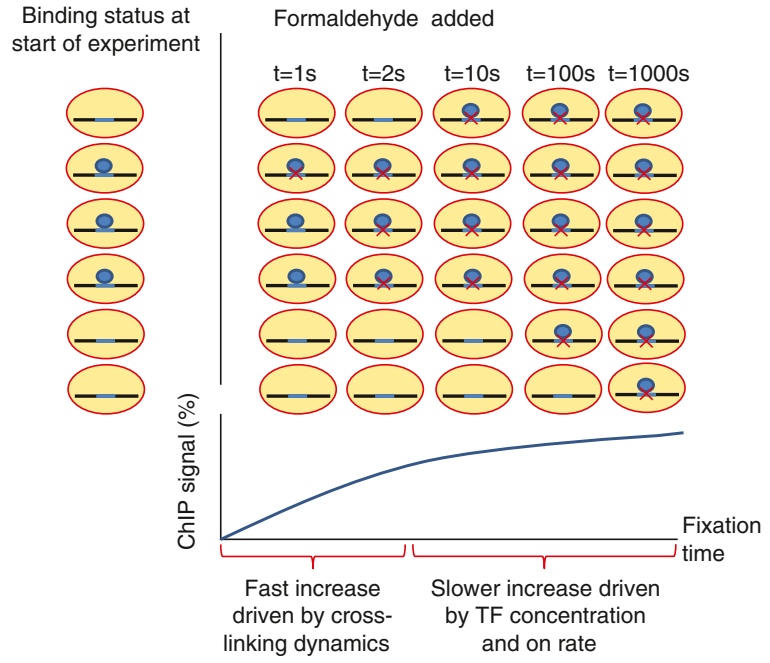

Figure 1 Overview of the cross-linking kinetic (CLK) method. Schematic showing a chromatin site (blue rectangle) interacting with a transcription factor (blue circle) in a population of six cells. Red 'X' symbols denote cross-linking. The plot shows how the site occupancy in the population and the resulting ChIP signal are predicted to change after addition of formaldehyde for varying periods of time. (Adapted from Poorey *et al.* [2]). ChIP, chromatin immunoprecipitation; t, time; TF, transcription factor.

Poorey and *et al.* conclude by using the CLK technique to study the action of one known regulator of TBP, the triphosphatase Mot1, that can dissociate TBP from DNA *in vitro* [3]. The authors measured TBP occupancy and dynamics at the *URA1* and *INO1* promoters in either wild-type (WT) or *mot1* mutant strains. In contrast to initial expectations, they found that TBP was more dynamic in the mutant strain. Additional analyses of TBP and TFIIB occupancies genome-wide in WT and *mot1* mutant strains suggest that Mot1 is responsible for dissociating weakly bound TBPs at diverse sites, thereby facilitating more-stable TBP binding in functional transcription complexes and promoting proper gene expression. This theory, warranting further experimental validations, highlights the importance of measuring quantitative kinetic parameters as a means to generate and discriminate between hypotheses.

Methodologies for in vivo measurements of chromatin binding kinetics

Currently, there are several methodologies for assaying the stability of *in vivo* interactions between DBPs and DNA. In FRAP experiments, the DBP of interest is labeled by a fluorescent fluorophore, and its dynamic assembly to a target of interest is monitored by microscopy. The key advantage of this system is in its high sensitivity, allowing the detection of short-lived interactions, and its ability to generate time-course *in vivo* data at the single-cell level. However, this technique is limited in terms of its resolution of chromatin binding location and normally requires genetic manipulations of both factor and binding site. Also, being an imaging-based method, it is limited in throughput and restricted to specialized laboratories possessing the appropriate experimental equipment and analysis tools [4]. This is in contrast to ChIP, which provides site-specific data on native DNA sequences, does not require genetic manipulations of the examined cells and is scalable to interrogate the entire genome in a single experiment. As such, ChIP is a common procedure in many laboratories.

Owing to the appealing properties of ChIP, several attempts were recently made to modify standard ChIP protocols to enable the extraction of binding kinetics. One such example is the 'competitor ChIP', in which two copies of the interrogated DBP are labeled with different epitopes. One copy is driven by the endogenous promoter, whereas the other (the competitor) is driven by an inducible promoter. During the experiment, the inducible DBP is activated and the replacement of the natively expressed DBP by the competitor at different loci is monitored by means of ChIP utilizing antibodies against the different epitopes [5,6]. In the work by Poorey and colleagues [2], a different method for extracting kinetic parameters is presented, by varying the time of formaldehyde fixation. One appealing property of this method is that it requires no genetic manipulations and should thus be readily applicable to any organism of interest. As in all ChIP-based methods, the results cannot be interpreted in terms of the fraction of the cell population that is bound by a specific DBP as the efficiency of cross-linking, fragmentation and immunoprecipitation can vary from site to site and between factors. As this field continues to evolve, comparing results from all methods for the same DBPs should provide important information as to the strengths and limitations of each.

The CLK method joins a body of recent work in the field of chromatin in which modifications have been introduced to long-established protocols to improve their accuracy and advance our quantitative understanding of DNA binding [5,7,8]. These studies make it evident that protocols should not be fixated and that altering existing protocols can lead to advances in biology. It is intriguing to speculate what other biological properties are hidden at our fingertips in the ordinary and routine experiments that we perform daily.

Abbreviations

ChIP: Chromatin immunoprecipitation; CLK: Cross-linking kinetic; DBP: DNA-binding protein; FRAP: Fluorescence recovery after photobleaching; GFP: Green fluorescent protein; $t_{1/2}$: Half-life; TF: Transcription factor; WT: Wild type.

Competing interests

The authors declare that they have no competing interests.

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