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Catching a glimpse of nucleosome dynamics

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Of the thousands of genes found in eukaryotic genomes, only a subset are expressed in a given cell type, and the composition of this subset underlies each distinct cellular phenotype. A major goal of genetics is now to understand how genomes are programmed to have a certain gene expression profile—in other words, to define the “epigenome” and to understand its operating principles. It is now clear that understanding the epigenome requires elucidation of processes that alter the chromatin fiber. The cast of characters responsible includes sequence-specific DNA binding proteins, the general transcription machinery, enzymes that add and remove chemical groups from DNA and chromatin, histone variants and nucleosome remodeling complexes.¹ Although many components of the epigenome have been identified, how they work together to program and perpetuate genome output remains an unsolved problem.

Much work in the field to date has focused on genome-wide mapping of nucleosomes, histone post-translational modifications (PTMs) and histone variants. These characterizations have been very fruitful in teaching us about the roles of these chromatin alterations in modulating histone-DNA interactions and recruiting secondary effector proteins to regulate gene expression.² However, such chromatin maps provide only a static snapshot of chromatin at a given instant, and an accumulating body of evidence indicates that chromatin is actually dynamic, with proteins constantly associating and dissociating, and nucleosomes being disassembled, reassembled and relocated. In fact, evidence suggests that the control of nucleosome eviction and assembly, or turnover, is a key element of epigenetic control in that it can regulate access to

DNA by transcription factors and other sequence-specific binding proteins.³ Thus, a more direct approach to understanding chromatin-based control of genome activity would be to examine nucleosome turnover directly, rather than mapping histone PTMs and variants.

The study of nucleosome turnover has been more technically challenging than PTM mapping, and until recently was limited to measuring the accumulation of inducible epitope-tagged histones into chromatin. To overcome this limitation, we developed a method for measuring turnover kinetics across the genome through metabolic labeling of histones. We call this method CATCH-IT, for covalent attachment of tags to capture histones and identify turnover.⁴ In CATCH-IT, cells are treated with the methionine analog azidohomoalanine (aha), which is incorporated into newly synthesized proteins, including histones. A biotin moiety is then covalently coupled to newly synthesized proteins through reaction of a biotin-linked alkyne with the azide group of aha.⁵ Nucleosomes containing newly synthesized histones are isolated with streptavidin beads, stringently washed, and the purified DNA is applied to a tiling microarray or subjected to deep sequencing (Fig. 1). The measurement of newly synthesized histone incorporation at each genomic location is then used to calculate the turnover rate of nucleosomes genome-wide.

By applying CATCH-IT to *Drosophila* S2 cells, we were able to estimate turnover rates across the genome and to gain new insights into the nature of epigenetic inheritance. We estimated turnover rates at binding sites for trithorax group (trxG) proteins, which maintain epigenetic regulatory elements in

an active configuration, and those for Polycomb group (PcG) proteins, which maintain silencing.⁶ Strikingly, turnover rates at trxG sites were higher than at sites of PcG protein binding. This suggests that trxG proteins maintain high levels of turnover, whereas PcG proteins act by reducing turnover and, therefore, net exposure of regulatory DNA. Our findings also indicated that nucleosomes at regulatory elements are reconstituted from new histones multiple times during a cell cycle, which would continually erase PTMs, calling into question the idea that histone modifications themselves transmit epigenetic information. Rather, epigenetic information could be based on regulated nucleosome turnover, which differentially exposes transcription factor binding sites. Thus, an emerging view is that histone PTMs and secondary effector proteins collectively dictate the intrinsic stability of a given nucleosome as well as its propensity to be remodeled. These characteristics determine how likely a nucleosome is to be disassembled or to change positions, and thereby expose the underlying DNA to sequence-specific regulators that directly control genome output.

Given that CATCH-IT uses a generic set of reagents, it can in principle be used on any cultured cell type. For example, changes in the epigenome that accompany cell fate specification can now be studied by applying CATCH-IT to stem cells and their differentiated descendants. Thus, as we begin to appreciate the epigenome as a dynamic entity, CATCH-IT and other methods that can capture kinetics will provide powerful tools for dissecting the relationship between chromatin dynamics and the perpetuation of gene expression states.

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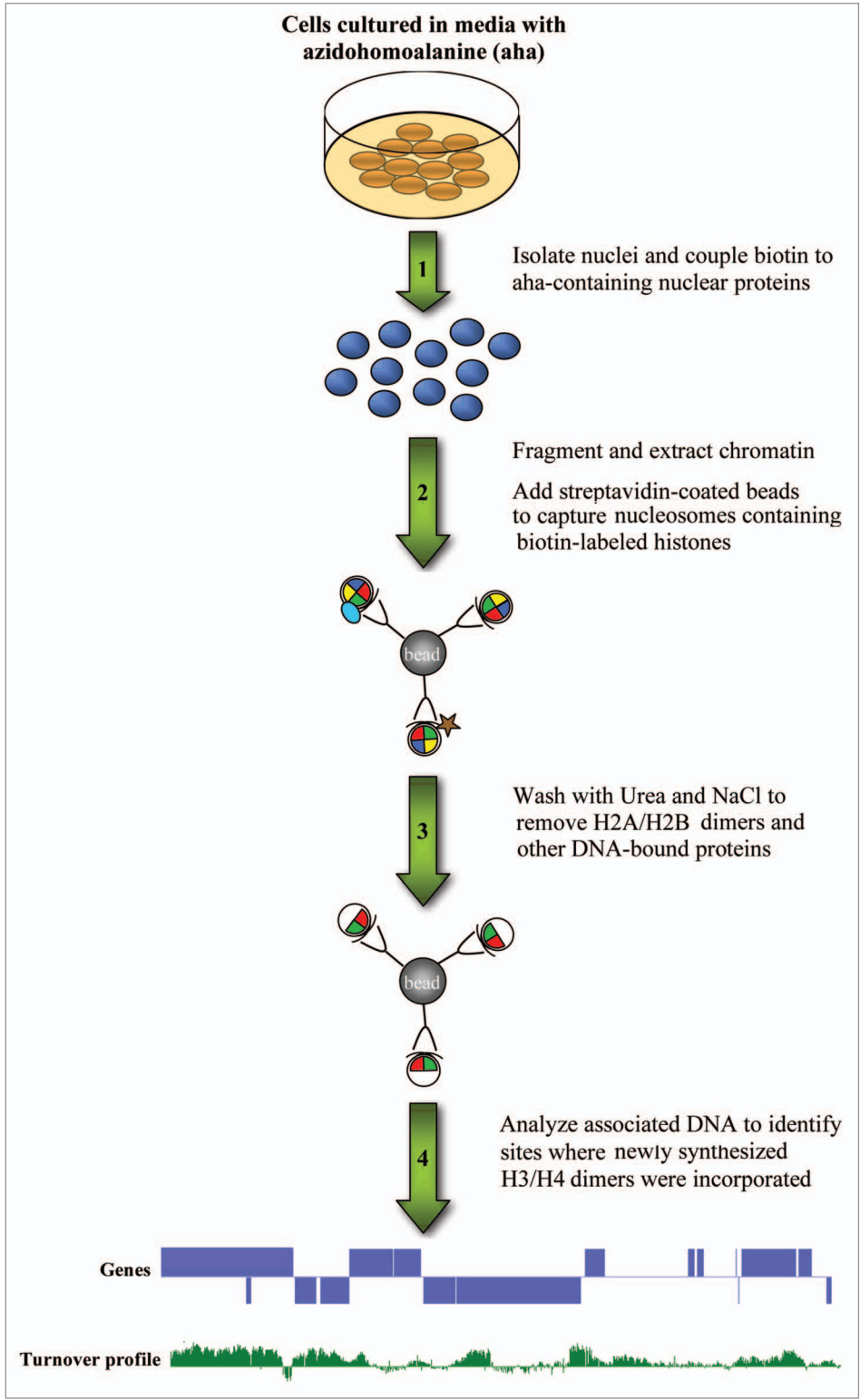


Figure 1. Schematic of the CATCH-IT procedure for measuring nucleosome dynamics. Cells in culture are first treated with the methionine analog azido-homoalanine (aha) for a brief period in order to label newly synthesized proteins. In step 1, nuclei are isolated from the aha-treated cells and biotin is ligated to aha-containing nuclear proteins through the copper-catalyzed cycloaddition reaction between the azide group of aha and an alkyne linked to biotin. In the second step, chromatin is fragmented to mononucleosomes with micrococcal nuclease, extracted from the nuclei, and streptavidin-coated beads are added to bind nucleosomes containing biotinylated histones. Step 3 involves washing the bead-bound nucleosomes with a solution of 4 M urea and 0.3 M NaCl to remove H2A/H2B dimers and other DNA-bound proteins, leaving only (H3/H4)₂ tetramers and associated DNA. This step is necessary because H3 and H4 are incorporated into the central tetrameric (H3/H4)₂ core of a nucleosome, whereas incorporation of H2A and H2B is into the flanking dimers. Therefore, only eviction and incorporation of new H3 and H4 represents turnover of the entire core particle, because the flanking H2A/H2B dimers can turn over without complete disassembly of the nucleosome. In the last step of the procedure, DNA that was associated with nucleosomes containing newly synthesized histones is isolated and analyzed by tiling microarray or deep sequencing. In this way the rates of nucleosome disassembly and reassembly, or turnover, across the genome can be estimated based on the extent of (H3/H4)₂ tetramer replacement at each site.

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