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Genome-wide kinetics of nucleosome turnover determined by metabolic labeling of histones

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Abstract

Nucleosome disruption and replacement are crucial activities that maintain epigenomes, but these highly dynamic processes have been difficult to study. Here, we describe a direct method for measuring nucleosome dynamics genome-wide. We found that nucleosome turnover is most rapid over active gene bodies, epigenetic regulatory elements, and replication origins in *Drosophila* cells. Nucleosomes turn over faster at sites for trithorax-group than Polycomb-group protein binding, suggesting that nucleosome turnover differences underlie their opposing activities and challenging models for epigenetic inheritance that rely on stability of histone marks. Our results establish a general strategy for studying nucleosome dynamics and uncover nucleosome turnover differences across the genome that are likely to have functional significance for epigenome maintenance, gene regulation, and control of DNA replication.

Nucleosome disassembly and reassembly, or turnover, is necessary for epigenome maintenance, but the mechanisms that are responsible remain unclear (1). One approach to this problem has been to map enrichment of the universal histone replacement variant, H3.3 (2-6), which requires complete unwrapping of DNA from around the histone core for its replication-independent deposition to occur. Genome-wide profiling of steady-state levels of H3.3 from *Drosophila melanogaster* S2 cells indicated that nucleosome replacement occurs most prominently across transcribed regions of active genes, and at promoters and binding sites of trithorax (trxG) and Polycomb group (PcG) proteins (2,3). Similar results were obtained for HeLa cells (7) and *Caenorhabditis elegans* embryos (8). A more direct approach, which can measure dynamics but is limited to yeast, is to express constitutive and inducible histone transgenes, and to measure the relative incorporation of their encoded tagged histones (9-11). These studies indicated that turnover rates were high at promoters and chromatin boundary elements but low within transcribed regions. Both approaches are limited by the requirement for transgenes and tags, and by the time lag during induction.

We have developed a general method for estimating the kinetics of nucleosome turnover that overcomes these limitations. Our strategy uses co-translational incorporation of the methionine (Met) surrogate azidohomoalanine (Aha) into proteins and subsequent ligation of biotin to Aha-containing proteins through the [3+2] cycloaddition reaction between the azide group of Aha and an alkyne linked to biotin (12,13) (Fig. S1A). To obtain nucleosome turnover rates directly, we treat cells briefly with Aha, couple biotin to nucleosomes containing newly synthesized histones, affinity purify with streptavidin, wash stringently to remove non-histone proteins, and analyze the affinity-purified DNA using tiling microarrays. We call this strategy 'CATCH-IT', for Covalent Attachment of Tags to Capture Histones and Identify Turnover. In

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Drosophila S2 cells Aha was incorporated into total protein, including histones, in a translation-dependent manner (Fig. S1B-D). Incorporation of Aha into newly synthesized histones increased in proportion to the length of Aha treatment for at least 3 hours.

To profile nucleosome dynamics across the genome, late log-phase S2 cells were starved for methionine for 30', treated with Aha for 3 hours, the biotin coupling reaction was performed on isolated nuclei, and chromatin was digested with micrococcal nuclease to yield mostly mononucleosomes. Biotin-tagged nucleosomes containing newly synthesized histones were then isolated using streptavidin beads, washed with a urea-salt solution to remove H2A/H2B dimers and other DNA-binding proteins (Fig. S2), leaving only (H3/H4)₂ tetramers (14), and the remaining DNA was labeled and hybridized to high-density tiling microarrays along with the corresponding input DNA. For comparison we also performed streptavidin pulldowns using chromatin from S2 cells expressing biotin-tagged H3.3 over a 2-day period as previously described (2). When array data for all genes with annotated ends were divided into quintiles by gene expression, aligned by gene ends, and log₂-ratios of pulldown DNA/input DNA averaged across genes, we found that CATCH-IT and H3.3 profiles were highly similar (Fig. 1A and 1C). In addition, CATCH-IT landscapes corresponded overall to steady-state H3.3 landscapes (Fig. 2, blue and green tracks), albeit with better definition of chromatin features. Such correspondence confirms that CATCH-IT depends on nucleosome dynamics, and we attribute the better resolution of CATCH-IT to its capturing turnover kinetics rather than steady-state replacement.

As a control, we also treated S2 cells with Met, rather than Aha, and obtained a featureless profile, confirming that the signals obtained with Aha represent newly synthesized histones (Fig. S3). We also found that independent biological replicates of the CATCH-IT procedure yielded similar results (Fig. S4).

We next asked whether CATCH-IT could measure nucleosome turnover kinetics. After treating cells with a 3-hr pulse of Aha, a sample was taken, and then cells were switched back to Met-containing media for a 1.5-hr chase, and both samples were processed as before. As expected, the chase resulted in an overall reduction of signal across expressed genes when compared to the pulse (Fig. 1A-B). We also compared the difference between the pulse and chase signals to the pulse signal on scatter plots using either all 2.1 million probes, or only genic probes from genes in each expression quintile. As expected for kinetic measurements, nucleosomes undergoing the highest turnover also tended to show the largest decrease during the chase, and this trend depended on gene expression level (Figs. 1D-E and S5). We conclude that CATCH-IT captures the dynamics of histone replacement. In addition, these results show that turnover across gene bodies is highly dependent on expression level (Fig. 1A-B). We suggest that the high level of turnover seen for gene bodies in *Drosophila* but low level seen for yeast (10) reflects biological differences between the two organisms in processes that evict or retain nucleosomes during transcription.

To observe histone incorporation into chromatin soon after treating with Aha, we removed successive samples 20', 40' and 60' after Aha addition. We observed that the histone incorporation landscapes generated at each time point were highly similar (Fig. 2 blue tracks), and this was confirmed by ends analysis (Fig. S6). We also examined incorporation at sites of epigenetic regulatory elements, as represented by sites of binding of the trithorax group (trxG) proteins GAF (GAGA Factor or Trithorax-like) and Zeste, and of the Polycomb group (PcG) proteins EZ (Enhancer-of-zeste) and PSC (Posterior-sex-combs) (15,16). Comparison of average incorporation profiles across GAF, Zeste and EZ+PSC sites showed that CATCH-IT identified these sites as regions of high incorporation relative to surrounding regions, with higher incorporation at GAF than at EZ+PSC sites (Figs. 3A-B and S7A-B). There was also

better peak delineation with CATCH-IT than observed for H3.3 or low salt-soluble chromatin, which represents classical ‘active’ chromatin (17) (Figs. 3A-D and S7).

We estimate kinetics based on modeling cycles of newly synthesized histone incorporation and nucleosome replacement, reporting the mean lifetime of histones as representing a single full cycle of turnover. We assume that Aha becomes incorporated into the histones occupying a particular site in the genome in direct proportion to the number of histone molecules at that site that have Met, and this number decreases as a result of turnover until saturation is reached (see SOM). This method will be insensitive to nucleosomes undergoing replication because the average turnover signal from a population of cells undergoing replication should be homogenous across the genome in unsynchronized cells. Therefore the presence of such a population in the culture should, at worst, only reduce the dynamic range of the microarray measurements. Also, this reduction is expected to be small, because it takes only a second or two to replicate through a nucleosome, $\sim 1/50,000$ th of the cell cycle, so that each nucleosome in the whole cell population undergoing replication will be diluted by $\sim 50,000$ nucleosomes that are not. Using the 20’ datasets from two independent experiments to calculate turnover rates (Fig. S8), we obtained mean lifetimes of ~ 1 hr for the peak just downstream of the transcriptional start site (TSS) of active genes, ~ 1 hr for GAF sites and ~ 1.5 hr for EZ+PSC sites (Table S1). These estimates are conservative, because any delay in incorporation of Aha would lead us to overestimate mean lifetimes. Therefore, nucleosomes within active genes and at epigenetic regulatory elements turn over multiple times during each ~ 15 -hr cell cycle.

We also compared CATCH-IT landscapes to binding sites for the ORC2 subunit of the origin recognition complex (ORC), which specifies replication initiation (18). We observed a striking correspondence, as exemplified by visual examination of a typical gene-rich region of the *Drosophila* genome (Fig. 2 magenta tracks). Although H3.3 levels also showed a correspondence to ORC levels (Fig. 2 green tracks), as previously observed (19), the resemblance of CATCH-IT profiles to ORC profiles was far more conspicuous. To better evaluate these correspondences, we aligned CATCH-IT, H3.3, and other chromatin profiles around the 5135 ORC peaks (19) and divided into quintiles based on ORC binding score (Fig. 3E-G). The nested peaks indicate a quantitative relationship between ORC binding and nucleosome turnover, suggesting that turnover facilitates ORC binding. In contrast, other chromatin features that would be expected for open or dynamic chromatin, including nucleosome density, mononucleosome/oligonucleosome ratios (a measure of micrococcal nuclease accessibility), and salt-soluble nucleosomes show little if any dependence on ORC abundance (Fig. 3H-P). Our findings support the hypothesis that replication origins are determined by chromatin, not by sequence features (19,20). Importantly, the better quantitative correspondence of ORC to CATCH-IT data than to other chromatin measurements implies that ORC occupies DNA that is made accessible by nucleosome turnover. In support of this interpretation, we note that very similar correspondences are seen when CATCH-IT data are aligned with GAF sites (Fig. S9), and GAF directs nucleosome turnover *in vivo* (21,22).

Our direct strategy for measuring nucleosome dynamics does not rely on transgenes or antibodies, but rather uses native histones and generic reagents. Thus, CATCH-IT provides a general tool for studying activities that influence nucleosome turnover. Using CATCH-IT we have found direct evidence that epigenetic maintenance involves nucleosome turnover, a process that erases histone modifications (10). The fact that EZ is responsible for di- and trimethylation of H3K27, but the nucleosomes that it modifies turn over faster than a cell cycle, argues against proposals that histone modifications required for cellular memory themselves transmit epigenetic information (23). Rather, by simply increasing or decreasing accessibility of DNA to sequence-specific binding proteins, regulated nucleosome turnover may perpetuate active or silent gene expression states and facilitate initiation of replication.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. CATCH-IT marks sites of histone replacement and reveals kinetics

A) Gene ends analysis of a CATCH-IT experiment from a 3 hour Aha treatment (pulse). All 9820 genes from FlyBase r5.13 with annotated 5' and 3' ends were grouped by gene expression quintiles (top 20% to bottom 20% based on GEO#GSM333845), aligned by gene ends, and the log₂-ratios of pulldown DNA/input DNA averaged across genes. B) Same as in (A) but with cells treated with Aha for 3 hours (pulse) followed by a 1.5 hour Met treatment (chase). C) Same as in (A) but for biotin-tagged H3.3 nucleosomes. D) Scatter plot showing the correlation between the pulse and the difference between the pulse and chase, using all 2.1 million probes on the array. E) Same as in (D) but using only genic probes from the highest and lowest gene expression quintiles.

Figure 2. Chromatin landscapes of CATCH-IT, ORC, H3.3, and salt fractions

Chromatin landscapes over a representative euchromatic region from a CATCH-IT time course of 20', 40', and 60' Aha treatment. Also shown are ORC binding, biotinylated H3.3 nucleosomes, and successive 80mM, 600mM and insoluble chromatin salt fractions (17). Genes are shown above chromatin tracks. Those above the line are the top strand and those below the line are on the bottom strand. Right panel shows a magnification of the indicated region of the left panel.

Figure 3. Kinetics of newly synthesized histone incorporation at epigenetic regulatory elements and sites of ORC binding

A) Average CATCH-IT time course signals over GAF and EZ+PSC binding sites. B) Same as in (A) but for a pulse-chase experiment with a 3-hr Aha pulse and 1.5-hr Met chase. C) Average biotinylated H3.3 signals over GAF and EZ+PSC binding sites. D) Average signals from chromatin salt fractions over GAF and EZ+PSC binding sites. E-P) Plots of various chromatin signals aligned at peaks of ORC binding and divided into quintiles by ORC peak score (19).